

nature**22 November 1979**

The Cambodian picture becomes clearer

In the past few weeks, the people of many countries have been exposed to the plight of Cambodia, or Kampuchea, through television programmes, through dramatic gestures, such as Mrs Carter's visit to Thailand, and through the widespread publicity of the relief agencies. The response has been generous; governments have pledged all manner of aid, and voluntary bodies are receiving a steady flow of funds. There is an evident need for the care of many thousands of Khmer refugees in Thailand. But as more is learnt about the situation within Cambodia, it is emerging that the need is not so much for immediate, massive emergency aid to relieve a national famine (for which there is little evidence), but for steady aid over the medium and long term to bring a once flourishing country back to health.

Observers who have recently been in Cambodia, or who have worked amongst the refugees in Thailand, are now beginning to bring back a more comprehensive picture, and this is augmented by satellite photographs of areas under cultivation. As far as food is concerned, it is likely that a substantial harvest was available at the end of 1978. Most of the country has one harvest per year, so food resources under normal circumstances should have been fairly adequate for most of 1979. Of course the circumstances are not normal and much of the stored rice could have been lost; possibly upwards of 200,000 tonnes of food aid has been shipped in this year at the request of the government but this should be put in the context of an annual consumption of about 1 million tonnes (assuming a population of 4.5 million). Where simple indicative tests such as the measurement of mid-upper arm circumference of children have been carried out, they equally suggest moderate under-nutrition rather than starvation.

The mid-term food supply in Cambodia depends very much on the 1979 harvest, which runs from October to December. Estimates of the amount of land under cultivation after the disruptions of the past year vary greatly, but the figures that are emerging suggest that less than a third of all arable land will be productive, and that the shortfall in food supplies for 1980 will be up to 50%; pledges through the World Food Programme and from individual countries will ensure that this deficit will be made up for the next six months at least, provided that movement through ports of entry can be expedited.

The problem on the medical side is, if anything, more serious. It

is reported that malaria is killing widely, and that untreated gastro-enteritis, tuberculosis and anthrax are also taking a heavy toll, although figures are proving hard to come by. During the dry season (until April) the incidence of malaria will fall, but concern is being expressed about the degree of drug-resistance being shown. Medical supplies have poured in beyond the capacity of local staff to use them and the problem for the immediate future is the development of a proper medical infrastructure to provide health care. With the (literal) decimation of the professional classes in Cambodia, no real progress towards self-sufficiency is likely until new schools are built, new people are trained and self-confidence is re-established.

The real need for Cambodia is not to relieve a nationwide famine; it is to supply food, medical aid and transport steadily and intelligently over the coming months, and for a large scale investment both in rural and urban re-construction and in the rebuilding of the professions. This poses difficult questions for the more thoughtful of those connected with relief. Film of starving children (and it cannot be denied that some children, particularly amongst the displaced, are starving), mention of widespread famine and hints of many further deaths in the coming weeks undoubtedly opens the floodgates of goodwill, but it also leaves relief agencies with strong political and emotional pressures to act on an emergency basis, even when it were better that investments were delayed until more information became available. There is a very serious dilemma here. Fund raisers know full well that tragic pictures bring in the money, but fund-spenders know equally well that the real needs are often elsewhere, or over a different time-frame, or for a different purpose. They know, for instance, that any money given now to alleviate the effects of the severe drought which has hit parts of India this year is ten times more valuable than cash that will flow in the summer of 1980 when the effects become all too visible.

The only resolution of this dilemma is slow, patient education of the public on the value of prevention. It is something that governments themselves should take on, if they see any merit at all in scientific assessments of disasters. The British government — now winding down its development education programme which had only been given a breath of life a year ago — could well make an imaginative step. □



Sociobiology critics claim fears come true

The National Front in England and the Club d'Horloge in France draw inspiration and support from human sociobiology, its critics said in Harvard last week. **David Dickson** reports.

HUMAN sociobiology came full circle last Thursday, when critics from Harvard University — the theory's principal *alma mater* — argued that its recent use by right-wing groups in Europe vindicated their earlier statements about its inherent political content.

A packed audience in the university's geology lecture hall was told that events in England and France supported warnings issued four years ago, when a biological interpretation of human social behaviour was suggested by Harvard zoologist E O Wilson in his book 'Sociobiology: The New Synthesis'.

The European experience "has realised some of our worst fears" said Dr Jonathan Beckwith, a geneticist at the Harvard Medical School. In England, for example, the extreme right-wing National Front party had used statements by Wilson and others in its literature to justify racism as a strategy for ensuring genetic survival. Similarly in Paris, right-wing intellectuals had used the writings of sociobiologists to defend claims about the inevitability of human hierarchies, said Beckwith.

In the US, sociobiology had appealed less to extremists than to those endeavouring to preserve the status quo against the claims of feminists or minority groups. But with the US entering "a new period of racial strife" extra caution was needed. "The European experience shows that groups who have used these ideas consider them to be powerful tools for rationalising their policies", said Dr Beckwith.

The Harvard meeting was organised by the sociobiology study group of Boston's Science for the People. Members of the group emphasised that they were not branding all those who wrote about sociobiology as extremists — but were drawing attention to the potentially dangerous connections between ideas expressed by scientists and the social and political climate in which they were propagated.

Thus two speakers warned that biological ideas about human nature had underpinned the practices of Nazis in Germany prior to and during the Second World War. Dr Stephen Chorover of the



National Front demonstration in Essex. NF literature has drawn on sociobiology texts.

Massachusetts Institute of Technology said that the roots of Nazi extermination policies could be found in ideas about 'social biology and racial hygiene' which arose in Germany in 1904, when a concern for the health of the individual factory worker evolved into a generalised concern to protect the public health against external threats.

Ideas about the 'naturalness' of social roles also permitted the Nazis to extol the role of women as housewives subservient to men, said Freda Salzman, a physicist from the University of Massachusetts.

Dr Richard Lewontin, Professor of Biology at Harvard, defended the scientific attack on human sociobiology. There was no evidence, he said, of a single human behavioural characteristic caused by genetic variability, as sociobiologists claimed. Labelling all critics of sociobiology as 'reds' was a "last refuge" which ignored scientific critics holding a variety of political views.

"The problem for the ideologues in our society is that they are unable to offer a reasonable excuse for continued inequality in a society which is claimed to be democratic" he said. Hence the temptation to label social inequalities as inevitable.

Dr Beckwith produced an article 'sociobiology; the instinct in our genes', which appeared earlier this year in the National Front publication *Spearhead*. The article says that scientists from the early eugenicists to modern sociobiologists have demonstrated that social differences have a biological basis, thus showing up "the genetic basis or racial awareness, discrimination and solidarity".

Similarly in Paris, said Dr Beckwith, right-wing intellectuals had recently gained wide publicity for their statements that hereditary differences posed insurmountable barriers to social equality. Members of groups such as the Club de l'Horloge used sociobiological arguments to support their claim that people with superior genes should become the leaders of society.

In both countries, the rise of right-wing groups resulted from economic difficulties. So far in the US, the use of socio-

biological ideas had focused primarily on sex roles. Newspaper articles said that men would always play a dominant role in politics because of their 'natural aggression'. Sociobiological influences were growing in school text books as an explanation of sex roles. "Exposing sociobiology for what it is an important part of the battle against racism and sexism in our society" said Dr Beckwith.

No sociobiologist had been asked to respond. But speaking from the floor, Dr Irvn Devore, Professor of Anthropology at Harvard, said that sociobiology was not being treated fairly by its critics — and that it was necessary to separate the scientific content from some of the claims being made for it.

Dr Devore agreed that the world-wide swing to the right was an "unhealthy movement", but denied that sociobiology should take any of the blame. "Historically it is unfortunate that this movement has taken place at a time when ideas about 'inclusive fitness' have emerged in biology that have revolutionised the study of animal behaviour. It is a question of whether these ideas are totally a product of that political/ideological shift or not. I do not think they are."

The critics remained firm. Dr Beckwith emphasised that the political uses of human sociobiology were inherent in its construction, not merely abuses of a neutral theory. Dr Lewontin said that the Chinese might refer to it in terms of the three vulgarities: "vulgar Darwinism, vulgar Mendelism, and vulgar reductionism." □



Oceanographers fear greater federal involvement

THE increasing activities of mission-oriented federal agencies in oceanographic research threatens to undermine a creative tension between government, industry and universities, according to a report published by a panel of the National Academy of Sciences.

The report also warns that a desire for "useful" research, coupled with possible research restrictions in foreign territorial waters, is shifting the emphasis from oceanography as a science searching for principles and understanding to oceanography as a tool with which to accomplish practical goals.

The report was prepared by an *ad hoc* committee established by the Academy's National Research Council to study the geological and geophysical research needs and problems of the continental margins. The chairman was Dr Alber W Bally of the Shell Oil Company.

Stating that a substantial increase in funding for research on domestic continental margins could produce considerable benefits in terms of both scientific knowledge and information about mineral resources, the committee says that it is disturbed by recent trends in the distribution of responsibilities between govern-

ment, universities and industry.

"Overall federal money for oceanographic research has increased substantially in recent years, but most new funds have gone to mission-oriented government agencies. Funding for basic oceanographic research done by scientists in academic institutions has barely kept up with inflation", the report says.

It suggests that government should concentrate primarily on research related to its function of governing and regulating the use of public resources — and that a considerable amount of future research effort should be delegated to universities.

The report also expresses concern about the possible impact of restrictions emerging from the Law of the Sea Conference, now under negotiation by the United Nations, under which scientists would require permission to conduct research within 200 miles of a country's coastline.

Given these restrictions, as well as the importance of scientific knowledge as a basis for resource exploitation, the report argues that "the time is ripe" to concentrate more research on domestic continental margins.

"Science — and particularly oceanog-

raphy — is international. However we believe that an effort with increased emphasis on national concerns is needed. We are recommending an increase in the momentum of solid-earth science research in our own back yard."

Commenting on the likely restrictions on international research, the report points out the irony that in recent years, pressure to demonstrate the social relevance of scientific research has caused university scientists to submit proposals containing rhetoric extolling the potential social impact of their work.

"Because of the emphasis placed on justifying research as having significance for resource evaluation, great strain may be placed on the credibility of the researcher if he proposes to do similar work on foreign margins, claiming it to be 'pure' scientific research" it says.

The report suggests that a strong academic domestic research programme on US continental margins might help to convince foreign sceptics that US academic institutions "are not implicitly interested in prospecting, that their interest is to contribute to the understanding of principles related to resources and to the environment." □

WHO looks at bacteria for vector control

THE potential of certain bacteria, notably strains of *Bacillus thuringiensis*, for the control of insect vectors of tropical diseases is being actively examined by the World Health Organisation, in collaboration with a number of laboratories on both sides of the Atlantic. For many years, strains of *B. thuringiensis* have been marketed by a number of pesticide manufacturers in commercial formulation for use against the larvae of various lepidopterous pests, but it is only recently that the potential of the serotype H.14 (also known as *B. thuringiensis israeliensis*) has been fully appreciated. This strain is now known to be extremely lethal for the larvae of certain Diptera, including mosquitoes of the genera *Aedes* and *Culex* which are vectors of malaria and filariasis respectively (although somewhat less so, apparently, against *Anopheles*). Now WHO sees the possibility of using it to control the blackfly, *Simulium damnosum*, the vector of onchocerciasis (river blindness).

On the commercial side, development is already going ahead, notably on the part of the Franco-Belgian firm Bellon-Biochem. They are working in close collaboration with the Pasteur Institute, where Dr Huguette de Barjac leads a team in this field. The first results from serotype H.14 formulations were so encouraging that Biochem decided to go straight to field testing, thus leap-frogging the lengthy procedure in a way that WHO itself could never do. The results against blackfly larvae in the Ivory Coast have so far shown

very high toxicity rates, with, a report states, "nearly total short-term innocuity against the associated non-target fauna".

The nature of the new material, however, has raised problems in its large-scale application that have yet to be resolved. The larvicidal action, it appears, is due to crystalline toxic compounds present on the outer coating of the bacterial spore. These operate in fact as a chemical insecticide. Unfortunately they have a high specific density, tending to sink to the bottom in water, and one problem is therefore to find a formulation that will keep the toxic materials afloat long enough for them to reach the target larvae downstream of the point of application. This in turn raises difficulties in attaining the concentration necessary to achieve the experimentally predicted results.

It is therefore necessary to put the maximum amount of crystal toxin into the primary powder used as a basis for the actual pesticide, and then to formulate the latter in such a way as to give the desired residual effect under normal field operating conditions. From the commercial point of view, the development of such a formulation could probably enable the manufacturer to patent his material, a matter of great importance in the highly competitive pesticide industry. Moreover, any such formulation must also pass the stringent requirements of most governments with regard to safety against mammals. This aspect is also being examined in a number of laboratories.

However, with government support in several countries — notably France, the USSR and Switzerland — it looks as though the new materials may reach a stage in the quite near future where mass field trials can be undertaken under WHO auspices.

The current resurgence of malaria, with increased resistance to normal insecticides as well as to therapeutics, and the pressure to find alternatives to such materials as DDT, will certainly encourage research in this field. Quite apart from its enormous potential for use against the vectors of malaria and other mosquito-borne disease, the new material could provide a valuable stand-by alternative to the pesticide Abate. The huge onchocerciasis control programme in the Volta Basin in West Africa at present depends entirely on this one pesticide for blackfly control.

Peter Collins.

France seeks bioengineers

France this year needs 30-40 biotechnologists familiar with such things as the purification of proteins, fermentation, and cell cultures. Next year there will be a need for 60-80 and the year after 120-160, according to a report reviewed last week by *le Monde*. The report is part of a massive work 'Sciences de la vie et société' prepared by Francois Jacob and available for consultation in Paris. □

European Science Foundation expands 'additional' activities

THE European Science Foundation, which over five years has established the role of 'stimulant' for European cooperation in basic research, is increasing its emphasis on 'additional', directly funded research. Approximately half of ESF's 10 million FF expenditure is now devoted to such work, and the proportion is increasing.

One example is a proposal for work in taxonomy, which the ESF believes is neglected in Europe — to the extent that assessment of environmental impacts (of SO₂ for example) are hindered. A 'European Floristic, Taxonomic, and Biosystematic Documentation System' should thus be established, and the work required would become an 'additional activity' of the ESF. This means that the 46 national research councils which are members of the ESF will be asked if they would like to contribute to the activity as an addition to their membership subscription to the Foundation.

Lord Flowers, in his presidential introduction to the 1979 annual report (to be published shortly) says: "It is through the additional activities that our work as a foundation will stand or fall". But he adds "we are conscious that each new activity adds to the financial burden of those of our members who participate in them".

For some additional activities, however, funds have been made available from outside sources such as the Leverhulme Trust and the Commission of the European Communities (which is supporting a study on the mobility and employment of scientists).

"Participation in the additional activities represents on the part of our member organisations a conscious and deliberate intention to give a certain priority to research which is conducted at a European level" writes Flowers.

The increasing importance of additional activities, which was hardly foreseen when the ESF was set up, may however lead to some difficulties among its members. They will be increasingly invited to fund projects controlled not from their own offices but from the ESF, testing their commitment to Europe. Thus, says the report, "money from an organisation in country A can and will be used to pay a scientist coming from country B, and possibly working in country C". Some members have already set up a new category in their budgets for 'European cooperation' to allow for this, says the report.

Another indication for the future of the Foundation was the agreement at this year's annual general meeting, held in Strasbourg earlier this month, that tentative approaches should be made to



Lord Flowers: promoting European activities

Soviet and East European scientific bodies to enquire if they might participate in some of the ESF's activities — without becoming official members. Lord Flowers will visit Moscow next year with this in mind.

Turkey's Scientific and Technical Research Council also joined the ESF this year, and Finland, announced it will become a full member. **Robert Walgate**

European synchrotron radiation facility brings governments together

IN AN unprecedented move, the European Science Foundation is to approach governments — albeit gingerly — to discuss the construction of a \$120 million European Synchrotron Radiation Facility.

Officially, the ESF will have nothing to do with governments, and preserves its independence jealously. Unofficially, the retiring secretary-general of ESF, Dr F Schneider, will in his retirement be convening an intergovernmental committee with the object of making the ESRF a reality. Thus the committee will strictly not be a part of ESF activities, although it is ESF-inspired.

Dr Schneider, who is retiring this year to be succeeded as ESF secretary-general by Belgian lawyer John Goormaghtigh, complains in an unsigned introduction to this year's annual report, that the lack of access to governments hampers the Foundation. The ESF's influence compared unfavourably with that of the National Academy of Sciences in the US, says the article. "A project proposed by the NAS is brought to the government for decision. The National Aeronautics and Space Administration's space science budget is decided in one place, not fifteen capital cities."

The report recommends a new role for the ESF: "In cases where concerted action is needed, a new machinery enabling the

Foundation to approach governments in the name of all its members [national research councils] and to act for them ought to be developed".

The ESF has prepared the case for the facility in two booklets produced by Yves Farge, director of the Laboratoire pour l'Utilisation des Rayonnements Electromagnétique (LURE), Orsay, and collaborators. The booklets are titled "ESRF: the feasibility study" (pp67) and "ESRF: the scientific case" (pp173) (May 1979) available from H Schmied, European Science Foundation, 1 quai Lezay-Marnésia, F-67000 Strasbourg, France. □

European Space Agency "crippled space science in Europe" says ESF report

WHEN the European Space Agency was established, funds for scientific research were confined to no more than 12-15% of the whole budget, to make room for an applications programme and the development of Ariane (ESA's own launcher, due for test flight in December) and Spacelab. "It is now apparent that this was wrong" says an independent report just published by the European Science Foundation. The chosen level of funding "seriously crippled space science in Europe", leaving European space scientists with only one sixth of the resources available to US scientists.

"Launches of ESA satellites are so infrequent" claims the report "that each hardware-building institute in Europe has approximately two chances in ten years" of participating in an ESA payload. "This is utterly inadequate, because the programme is so small that new talent is not likely to be attracted". Some 1500-2000 scientists in the countries of ESF members are presently using the results of space investigations, the report estimates.

Financial pressures have also limited ESA's collaborations with the National Aeronautics and Space Administration; ESA's contribution to the space telescope, for example, is only 15%. Europe should aim to match the US and the USSR in space science, says the report. An annual ESA launch rate of one satellite fully instrumented by European groups, and "additional spacelab flight opportunities" will be required to keep European groups above "the critical threshold of efficiency".

The report recommends that ESA increase its mandatory scientific budget. But this requires unanimous approval by all ESA member states, and is therefore unlikely. Optional programmes could augment the mandatory budget, however, because they allow funding to be more flexible, says the report. "Space Science in Europe" (pp66) available from H Schmied, ESF, 1 quai Lezay-Marnésia, F-67000 Strasbourg, France. □

"Hidden cuts" will damage UK medical research

THE Medical Research Council, like other UK research councils, has had to face "unprecedented" and immediate cuts in its current budget, as a result of the present British government's policy of reducing public expenditure. But although these amounted, in the MRC's case, to a £500,000 reduction in direct funding from the Department of Education and Science, "hidden cuts" would hit the MRC harder, said its secretary Professor J L Gowans at a press conference last week.

These were losses through having to meet pay increases from within a falling budget, through having to pay higher value added tax (the rate has been increased from 8% to 15%). And the tropical disease research programme of the MRC has been badly hit by the reduction in budget of the Ministry of Overseas Development, when it was absorbed into the Foreign Office to become the Overseas Development Administration. The total cuts amounted to some £2.1 million, or 3.5% of the MRC budget, said Professor Gowans.

Furthermore there was no firm information available to the MRC on which it might make plans for 1980-1 and beyond (though Professor Gowans expected that it would sustain further cuts). This caused great difficulties "as the shortest period for which we have to plan funding is three years, the duration of a research grant".

"We need a stable budget both from the DES side, and from the universities." The dual support system, whereby the universities themselves provided the basic facilities for a laboratory — building, stores, technicians and so on — was creaking.

Universities were also sustaining cuts, and in endeavouring to maintain teaching

were leaving more and more of the basics of laboratory funding to the research councils. The system was "creaking under the strain".

"If in addition to the cuts we took this year, there are cuts of any magnitude in successive years, then damage will be done to medical research in Britain . . . There is a danger that high class new applications for research will go unfunded."

However, despite the cuts the MRC managed to set up three new research units in the past year: a molecular haematology unit in Oxford, one for neuroendocrinology in Newcastle, and an environmental epidemiology unit in Southampton.

The council's annual report for 1978-9, just published, pays attention to the jobs problem facing medical researchers. The MRC, which employs 991 clinical and non-clinical research scientists, "is constantly

aware of the need to keep under review its career structures in relation both to the interests of the individuals themselves and the advancement of science" says the report. The report offers no panaceas.

●The Advisory Board to the Research Councils, which coordinates the direct funding of the councils through the Department of Education and Science, is consulting with the Treasury and the National Research Development Corporation to see if there can be any financial return to the Councils from patents arising from research council funded work. At present all benefits from these are retained by the NRDC, a large fraction of whose income comes from patents on the 'cephalosporin' antibiotics discovered under MRC funding. The talks, however, are progressing very slowly.

Robert Walgate

Commons debates animal experiments Bill

Two separate bills to control the use of animals in scientific experiments are currently being considered by the UK Parliament: Lord Halsbury's Animal Protection Bill (Nature, 16 August page 534) which successfully received its second reading in the House of Lords last month, and Mr Peter Fry's Protection of Animals Bill which was read for a second time in the House of Commons last week.

Both bills are seeking to up-date existing legislation which is based on the 1876 Cruelty to Animals Act. "Whole new procedures have been developed, which I understand are totally outside the Act's jurisdiction", said Peter Fry during the opening speech to last week's commons debate. For example, the transplant of tumours from one animal to another and the implanting and developing of antibodies within animals, he said, were procedures now in use which were not covered by the Act. New legislation was needed which would incorporate these and other procedures developed over the past 103 years.

Peter Fry's Bill goes further than Lord Halsbury's in several respects. In particular, it lays greater emphasis on the need to reduce the number of animals in experiments by controlling the type of research that is done. Like Lord Halsbury's Bill, it recommends that alternatives to animal experiments should be used wherever they are available but it also emphasises the need to eliminate repetition of experiments.

One of the more controversial clauses of the Bill, however, states that "operative procedures" will only be allowed for "the advancement of biological science" in a way calculated to save or prolong human or animal life or relieve suffering. Is it possible to decide in advance what research will lead to such progress asked Tam Dalyell, who cited the discovery of enkephalin as an

example of research with animals of far reaching significance which could not have been anticipated. Mr Fry accepted the point, which had also been made to him by the Medical and Agricultural Research Councils, and suggested that the relevant clause be amended "to satisfy that kind of objection".

Mr Fry's Bill also differs substantially from Lord Halsbury's over the granting of licences to experiment with animals. It recommends four different grades of licence covering all types of procedure. Grade 1 licences, explained Mr Fry, would be for the simplest procedures, such as giving injections. A Grade 2 licence would be the equivalent of the single licence now existing under the current legislation. It would allow surgical procedures under anaesthesia from which the animal is not allowed to recover. A Grade 3 licence would cover surgical operations where the animal is allowed to recover afterwards provided that it is suitably anaesthetised throughout the entire procedure. And a Grade 4 licence would be granted for "research in which pain is inevitable as part of the experiments". Mr Fry expects that this latter category will raise the most objections from the antivivisectionists.

The Bill had a successful second reading and now goes forward to the committee stage. However, Mr Timothy Raison, Minister of State, Home Office, reminded MPs that a European Convention on animal experiments was currently being drawn up and that it would be wise to wait for that before passing legislation. The government, he said, could "not regard the Bill as it stood as an acceptable way of dealing with issues of a fundamental nature concerned with animal experimentation". Nevertheless, it would not stifle the debate by opposing the Bill at this stage.

Judy Redfearn

Hubert Curien to head ESF in 1981

PROFESSOR Hubert Curien, 55-year-old President of the French Centre National d'Etudes Spatiales, is to succeed Lord Flowers as President of the European Science Foundation in 1981. Flowers, now Rector of the Imperial College of Science and Technology, London, was founder-President of the ESF and will have served two three-year terms when Curien takes over.

Professor Curien has played a prominent role in science politics since 1966, when he became scientific director of the Centre National de la Recherche Scientifique, the body which funds much of France's basic research. "A laboratory is not a laboratory in France without some CNRS staff" says Curien. In 1969 he became its Director-General. In 1973 he was délégué general to the Délégation Générale à la Recherche Scientifique et Technique, and since 1976 he has been President of the CNES. □

news in brief

Academics call for removal of Boston University President: Scientists and academics at MIT and other institutions in the Boston-Cambridge area have called for the removal of John Silber as President of Boston University. A petition circulated by Dr Salvatore Luria, Nobel laureate, and Professor Emeritus of biology at MIT, condemns Silber's administration of the university as "notorious in the academic community and the nation at large."

The current calls for censure, described by the petition as "the latest in a pattern of violations of civil liberties", have arisen from the threat by Silber to dismiss or suspend five tenured professors who had, at the beginning of September, expressed their support for striking clerical workers at Boston University. In order not to cross picket lines, the five gave their lectures in places other than the assigned class-rooms. This, said Silber, constituted a breach of contract.

The petition was launched on 5 November. Within ten days, Silber was saying that the five professors could avoid disciplinary action if they submitted a written apology. He denied that the university had ever "threatened" dismissal or suspension although these options were not, he said, "ruled out". Some milder form of sanction, such as reprimand or docking of pay was, however, possible.

USSR has no oil crisis: A new Swedish report, sharply disagreeing with current CIA estimates, claims that Soviet oil reserves are 100% larger than current Western estimates allow. The report, by Petro Studies, an independent Swedish oil research firm specialising in analysing Soviet oil and gas industries, contradicts recent conclusions made by other Western analysts. Using a systematic literature search of all Soviet information released over the past 20 years, the report says that the "large underestimation of the USSR reserves by Western analysts requires that the world's oil reserves must be revised upwards by an amount equalling the combined proved reserves of the US, Canada and Mexico." The report claims that the USSR has been the world's leading producer since 1974 exceeding the US by a factor of four and Saudi Arabia by 25% and that it will increase its oil exports to the West in order to earn hard currency. "There is no danger at all that the USSR will become a net importer of oil in the next ten years at least and it will not compete with other nations in the purchase of OPEC oil." *Soviet Proved Oil Reserves 1946-1980*. PetroStudies Co. Sjöblads väg 27, S-21370 Malmö, Sweden.

UK nuclear information restricted since Three Mile Island: An official internal memorandum advocating tight control over the amount of nuclear reactor information to be allowed to reach the public was circulated among high officials of the Central Electricity Generating Board last April shortly after the Three Mile Island accident. The memorandum, leaked to the *Guardian* on 19 November, was signed by Mr Roy Matthews, the CEGB's director of health and safety, and advocates a two step process for dealing with telephone calls from the "nuclear opposition". Step one is to refuse to provide the information and step two, in the event that the caller persists, is to request a written question which "then can be considered centrally and if it is agreed that answers should be given then we will have to insure that this is done on a consistent basis."

Windscale widow awarded £67,000: The widow of a former worker at British Nuclear Fuels Ltd's Windscale nuclear plant was awarded £67,000 in a Crown Court settlement last week. The case was brought by the General and Municipal Workers Union on behalf of Mrs Bridget McAreavey after her husband, Mr Malcolm Pattinson died of myeloid leukaemia at the age of 36. Pattinson worked as a process worker and general worker at Windscale from 1957 to 1970. He died on 28 May 1971. The case was the first in the UK in which a company has admitted liability for radiation caused injury or death. (In a similar case in 1976 British Nuclear

Fuels agreed to an out of court settlement of £22,500 to the widow of Jonathan Troughton and £8,000 to the widow of Harry King, both of whom died of leukaemia after working at Windscale.) An official BNFL statement said that the "settlement has been reached on the particular facts and issues in this case and should not be considered prejudicial for any future claim, pending claim, or past claim." David Gee, national health and safety officer for the GMWU was "relieved" at the action but was "not pleased" at the length of time it took to reach a decision. "These court room scenes are quite stressful and we have pressed BNFL for an automatic compensation scheme since the Troughton and King settlement in 1976. BNFL agreed to consider this but there has been no response so far. We have 30 workers at Windscale who have received maximum permissible lifetime doses and we need a protection of earnings scheme for them. Someone who is made redundant at 35-40 has a long way to go and their level of skilled wages must be protected."

ESA-NASA to launch Halley-Tempel 2 comet mission: The European and US space agencies, NASA and ESA, are seeking experimental proposals for a planned mission to two comets, Halley's and Tempel 2. The mission has been timed to take advantage of the once every 76 year appearance of Halley's comet during the winter of 1985-1986 and will last four years. NASA will be responsible for the launching and mission operations and ESA will be responsible for the Halley comet probe system.

US Army seeks new funding for nerve gas weapons: The US Army is again seeking funds for a facility to produce nerve gas weapons, halted since the early 1970s following widespread protest against the use of chemical weapons in Vietnam. Last year President Carter rejected a request from the Army to start construction of such a facility, which would produce so-called binary shells containing two separate nonlethal chemicals which combine to form a nerve gas after the shell has been fired. A report in the *New York Times* last week stated that the Army had received "tentative approval" for its request, which it has resubmitted for inclusion in the 1981 budget. However, the report also says that administration officials are split over whether the decision to proceed with the production of nerve gas weapons would alarm other member countries of the North Atlantic Treaty Organisation, where such weapons are most likely to be used in combat.

Although President Carter turned down the request last year, his budget proposal included funds to continue development work on the weapons.

Johns Hopkins gets space telescope institute: A group of US universities responsible for running a number of major US research facilities, has proposed that the Space Telescope Science Institute planned by NASA should be located at Johns Hopkins University in Baltimore, Maryland. The recommendation was made last week by a board of directors of the Association of Universities for Research in Astronomy (AURA) which is preparing to submit plans to NASA for an institute to direct space telescope research on the university's Homewood campus. The 2.5m diameter telescope is scheduled to be launched from the space shuttle in late 1983 and to operate for at least 20 years. AURA selected Johns Hopkins after examining offers to host the institute from seven separate universities.

Weiss appointed director of Institute of Cancer Research: Robin Weiss, aged 39, presently head of the Laboratory of Viral Oncology at the Imperial Cancer Research Fund, has been appointed Director of the Institute of Cancer Research in London. The ICR is a major European cancer research organisation with a staff of 450 and an annual budget of £5 million. Weiss' appointment is seen as a move by ICR to strengthen its basic research approach to cancer research.

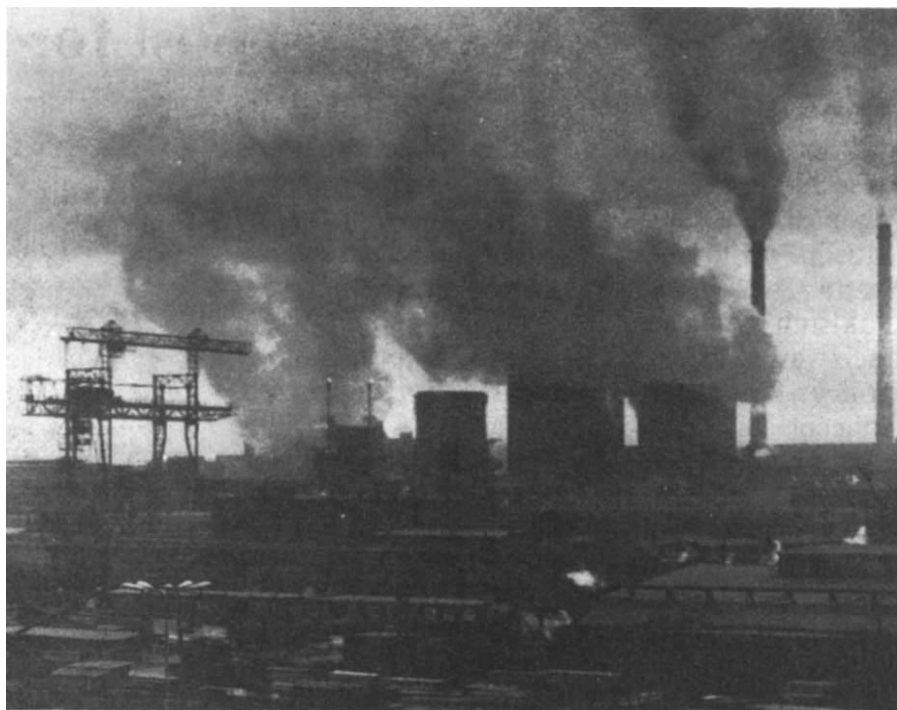
East Germany may debate pollution

LAST week, the Mecklenberg Synod of the Evangelical Lutheran Church issued a call for public discussion on the dangers of nuclear energy — the first time that the issue has been raised in the GDR. The church in East Germany is not often outspoken on matters outside its immediate concern, and the Mecklenberg proposal for talks between politicians, scientists and churchmen on the nuclear issue is unlikely to receive unqualified official approval.

Judging from the listed "priorities" for science and technology in the current five year plan, environmental issues are not a prime concern of the East German planners, except perhaps as regards water resources which are extremely scanty (2,100 m³/year per head of the population, according to 1970 Comecon figures, as against the Soviet Union with 19,600 m³/year, Bulgaria with 24,000 m³/year, and Hungary (12,000 m³/year per head).

Conservation, to the East German planners, seems primarily to mean energy saving — as exemplified in last week's address of State Secretary Ziergiebel to the "Urania" society for the dissemination of scientific knowledge. The future problems of the energy industry are enormous and are by no means easy to solve, he said. Energy saving, in the present world situation, is "a principle of socialist economic management", which, by the use of effective technologies, can effect, as in the case of the Leuna combine, "a million-fold saving".

The Magdeburg appeal, however, seems



'Walter Ubricht' chemical plant, Halle, East Germany: effluents as well as profits

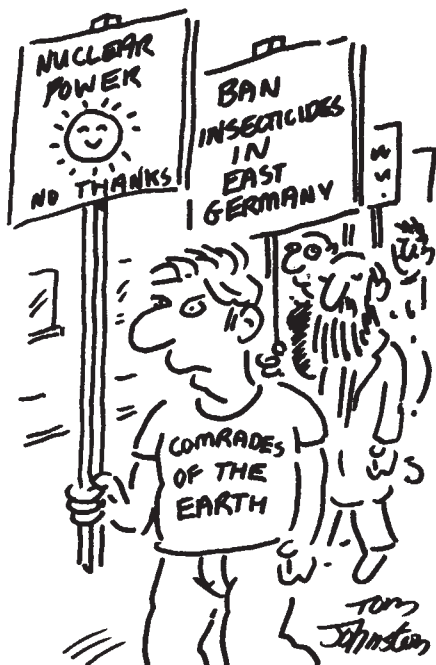
to reflect a wider concern with environmental issues among the East German public at large. The GDR is a notoriously difficult country in which to gauge public opinion — unauthorized communication of information to foreign journalists can merit a 12-year jail sentence. However, a recent "exchange of letters" in the prestigious philosophical journal *Sinn und Form* may well serve as a pointer.

In the first of the letters, Gunter Kunert, a well-known writer, addresses the editor on the dangers inherent in modern technology. He ironically dismisses the "class-standpoint" that science and technology in the hands of "progressive forces" never have the same negative consequences as occur under capitalism". To take but one example, in "industrialized agriculture", "we have a metaphorical tiger by the tail", yet, in spite of the risk of poisoning, "agriculture will get nowhere without insecticides and pesticides", and to abandon them would result in world famine. Quickly he runs through a "catalogue" of possible disasters more familiar to a western than East German readership: the possibility of climatic change through CO₂ buildup and the ensuing melting of the polar ice-caps, destruction of the ozone layer by aerosol propellants, the hazards of nuclear energy — in the GDR, he said, all these dangers inherent in the development of civilization are put out of mind. DDT is banned, but new substances, whose effects are only vaguely known, are spread on the fields. No one is concerned with long-term hazards, and people simply proceed on the principle that "the human race will survive — it always has so far!"

Kunert's letter is a moderate and reasoned appeal, not for the abandoning of technological advance, but simply to a greater awareness of long-term and cumulative effects. His stance on pesticides, for example, seems very similar to the attitude of Hungary's plant protection officers. To Wilhelm Girnus, Editor of *Sinn und Form*, however, Kunert's proposals seem tainted with western doomwatch. Girnus does not, of course, dismiss environmentalism altogether; in his reply, more than three times the length of the original letter, Girnus mentions, though with some doubt, work done on the smoke-pollution of pine-trees, and even waxes enthusiastic over the Soviet Union's integrated plans (which include conservation) for the region to be spanned by the new "Baikal-Amur-Mainline".

However, his major task is dialectic. From an argument which ranges from the content of socialism to the views of a "certain Herr Schicklgruber" on the vocabulary of "Jewish liberalism", the message emerges; a doomwatch mentality, always on the lookout for "black possibilities" can lead only to "manic sweeping judgements", helplessness, and moral paralysis. In the capitalist world, he says, "a kind of addiction to catastrophic experiences" is becoming rampant. While politely agreeing with Kunert that the "warnings of a serious scientist about the dangers of a new discovery must be taken seriously", in his capacity as editor of a leading philosophical journal, Girnus makes the official standpoint abundantly clear. If citizens of the GDR do want to discuss ecology, they must eschew the doomwatch edition.

Vera Rich



A revival for Islam, a boost for science?

TODAY is the first of the fifteenth Islamic century: a century in which Islam again is becoming a potent force in world affairs. **Ziauddin Sardar**, *Nature* correspondent for the Muslim world, recently visited eight key states — Iran, Turkey, Syria, Saudi Arabia, Egypt, Pakistan, Malaysia, and Tunisia — to report on their varied attitudes to science and technology policy. He found an increasing interest in the concept of an 'Islamic Science', a socially concerned science set within the philosophy of Islam. In the first of a number of articles which will appear in *Nature* over the next few months, Sardar here reviews opinion on whether science and technology can be so 'Islamised'. Next week he will discuss thinking in Iran; after that, nuclear power in Turkey.



Oil pipelines in Saudi Arabia: Islam's new technology

THE global middle belt: from the shores of Senegal and Morocco to the Pacific Ocean and the islands of Indonesia, and north to south from the Mediterranean coast of Turkey to Somalia; almost one billion people, constituting one-third of humanity, and speaking over 50 languages; 46 sovereign states at various stages of development and with ethnic backgrounds as diverse as those of Arabs and Afghans, the Housa and the Pathans, the Chinese and the Malays, welded together in a bond that goes back fourteen hundred years to this very day; the bond of Islam; the Muslim world.

Only three decades ago, the Muslim community was a subject community. The fifties saw the independence of many Muslim states. However, they soon learned that political independence alone is not enough. To be free and yet to be dependent on the departing colonial powers for scientific expertise, technology transfer and foreign aid is not real freedom. The Muslim world is now beginning to take the view that the post-independence development strategies occidental-style have been in error. This awakening is based on a strong self-assertion of the Muslim world's Islamic identity and brotherhood, and is seen by many as a new revival of Islam.

The Muslim world is going through a deep, although turbulent, process of self-examination and self-renewal. Almost everything is placed under the microscope and investigated from the perspective and world-view of Islam. Both western consumer materialism and eastern (dialectic) materialism are dissected with fervour. Serious attempts are being made to seek and pursue Islamic alternatives.

How would this sense of revival affect science and science policy in the Muslim world?

There is little doubt that most Muslim countries with science policies are re-examining and re-evaluating those policies. Iran, for example, is taking a very deep look at its science. Syria is reorganising its science with concern for the culture and values of its society. Iraq and Algeria are going through a process of 'Arabisation'. Attempts are being made, largely due to the efforts of the Jeddah-based

Organisation of Islamic Conference, to persuade Muslim countries without science policies to formulate down-to-earth policies based on their needs and requirements. An Islamic Foundation for Science, Technology and Development (IFSTD) has been set up by the Tenth Islamic Conference of Foreign Ministers, held in Fez, Morocco, in April 1979, to help and encourage the development of science in the Muslim world, draw programmes for co-operation and co-ordination of research activities amongst the Muslim countries, and help give a unified direction to science policies (see box).

The revival of Islam is also providing an impetus for science. Mohammed Ajaz al-Khattib of the University of Damascus thinks that this is a natural process. "The pursuit of science is an obligation for an Islamic society. If an Islamic society experiences an increase in the awareness of its Islamic identity, it will fulfil its obligation to pursue science with more vigour."

According to al-Khattib, for Muslim societies science is not a luxury, but a necessity. "Some 750 verses, almost one-eighth of the Qur'an, exhort the believers to study nature, reflect, make the best use of reason, and make scientific enterprise an integral part of the community life. In contrast, the legislative verses are only 250", he says.

Kalim Siddiqui, Director of the London-based Muslim Institute for Research and Planning, told *Nature*: "The new and invigorated civilization of Islam which is now re-emerging has a solid body of axioms that have stood the test of time without the help of 'reformation' or otherwise 'revised' or 'authorised' versions. The new civilization of Islam will be capable of boosting scientific activity and scientific knowledge to heights and achievements unknown to man before".

Zia Sardar is the author of 'The Future of Muslim Civilisation' published today by Croom Helm, price £10.95 (hardback) £4.95 (paper)

In the history of Islam, science occupies a central position. Although known to the West largely for its historical influence on the development of European scientific thought, it also had a powerful effect on the development of Muslim civilization itself. Islamic science is considered to be one of the basic ingredients that provided the drive for the "Golden Age of Islam". So it is now being realised that science is even more essential for a Muslim civilization of the future. This realisation is obviously related to the sense of revival that is sweeping through the Muslim world. There are, however, two special factors that have helped to create this scientific consciousness.

The first was the publication of Seyyed Hossein Nasr's book, *Science and Civilization in Islam* (Harvard University Press, 1968). This was the first serious attempt to examine Islamic science *within* the context of the civilization of Islam. But more than that, because Dr Nasr, an Iranian scholar now in self-exile in London, commanded attention and respect in the West, he made it possible for many a Muslim scientist, with the usual developing country background and sense of inferiority, to speak about Islamic science without feeling ashamed. His second book on the subject, *Islamic Science: An Illustrated Study* (World of Islam Festival Trust, London, 1976), took the discussion to the popular and student level. It is responsible for generating much excitement and discussion on the campuses of the universities of Muslim countries.

The Islamic Solidarity Conference on Science and Technology, sponsored by the University of Riyadh and held at Riyadh's ultra-modern King Faisal Conference Hall in March 1976, was the second catalyst (see *Nature* 260, 663; 1976). The conference spent a great deal of its time soul-searching and discussing "Islamic ways of doing science". At the conclusion of the conference it was declared that the future of the Muslim world is closely linked to the revival of Islamic science and the operationalisation of its concepts and principles.

That was in 1976. Since then much discussion has ensued on the nature of Islamic science. At the same time, there have been a number of attempts to 'breathe the spirit of Islam in natural sciences' and to 'Islamise' the teaching of science. Notable amongst these are the innovations concerning the teaching of 'Islamic technical subjects' at King Abdul Aziz University, Jeddah; various attempts at "Arabisation of science" in Syria, Iraq and Algeria; and the entire reorganisation of education and the radical approach being planned and developed to the teaching of science in Iran.

Much of the discussion on Islamic science is woolly and confused. This is only natural. In its practical form Islamic science existed in a distant past and the Muslim civilization has been out of touch

with science for over four centuries. During this period the Muslim civilization degenerated to reach its nadir, was consequently colonised, and has only recently reemerged to face what are probably the biggest fundamental material changes human life has ever experienced. Furthermore, there is an acute poverty of analytical and historical literature on the nature and style of Islamic science.

Four points of view

The discussions on Islamic science centre around two basic questions: what is Islamic science? And how does it differ from the practice of conventional science? Some Muslim scientists, however, are asking an even more fundamental question: Is there such a thing as Islamic science?

Four points of view can be distinguished on these questions. The first is what we may call the traditional (western science) ethos. The second and third viewpoints maintain that Islam has a great deal to say about science, but are divided on where exactly Islam enters the picture. The fourth argues that Islamic science is something quite different from science as it is practised today.

Ali El Hili, Dean of the Faculty of Mathematics, Physics and Natural Sciences, University of Tunis, presents the traditional view of science simply and forcefully: "there is only one science: it is universal, neutral and value-free. The study of nature is impersonal and free from human values. It *has* to be that way", he says. Discussions of Islamic science worry him. "We cannot compromise the basic rationality of science with our religious concerns". And he warns: "if we compromise the basic objectivity and neutrality of science with Islamic values and ethics we will destroy the very foundations of science".

Tevfik Karabag, Secretary-General of the Scientific and Technical Research Council of Turkey, agrees with Hili. "Science is universal", he argues, "but a value-judgement may be applied in giving emphasis and pursuing a particular area of science". Thus the Muslim countries may choose not to pursue research on fermentation due to restrictions on alcohol in Islam. However, the choice of undertaking or not undertaking a particular area of research is available only in applied fields. "Basic scientific research, in all areas, must be pursued for its own sake", says Karabag. If one were to talk about Islamic science in Turkey today, he says, one is likely to be ignored and isolated by one's colleagues and labelled a "fanatic".

There is no such danger in Egypt. Fahamy Ramadan, General Secretary of the Cairo-based National Research Centre, who spends most of his free time reading the Qur'an, declares unambiguously: "science has no home, no religion, no particular bias. Everyone contributes to it, irrespective of race, creed or culture". He



Ancient Islamic Science: an astrolabe

mourns the sorry state of science in the Muslim world and is convinced that "the road to the future of science in the Islamic world lies through Arabisation". "This is the link between our past and present", he says.

Fahamy argues that science and technology will not settle in "Muslim homelands" unless Arabic becomes, for the Muslim people, the language of science. "Only through Arabic can we encourage cultivation of sciences in the Arab and Islamic countries, encourage science to become a genuine component of Arab and Islamic thought, and assimilate the love of science in the mentality and minds of our citizens", Fahamy says.

Many Muslim scientists would agree whole-heartedly with him. Indeed, serious attempts are being made to Arabise science in Iraq and Syria. But there are a large number of Muslim scientists who argue that the issue of Islamic science is deeper than simply doing and teaching science in Arabic.

Secular or Islamic approach?

The second position on Islamic science is articulated by Affin Suhaimi, Dean of the Faculty of Science and Environmental Studies, University of Agriculture, Selangor, Malaysia. "Science is neutral", he says, "but the attitude by which we approach science can be secular or Islamic". What is the Islamic approach to science? "We recognise the limitation of human reason and human mind; and we acknowledge that knowledge is the property of God", explains Suhaimi.

"This knowledge is granted to us as a trust. We can use it for the benefit of society or we can put it to destructive use. Either way, we as scientists, are responsible before God for the use of that trust", he says.

Like Hili, Suhaimi too is concerned about the discussions on Islamic science. The argument that Islamic science is

something different from western science, complains Suhaimi, is sometimes used to block the transfer of scientific knowledge from the West to Muslim countries. "Science, in its pure, value-free form, we can take from all sources", he argues. "We should take from the West as much as we can". To support his argument he quotes a tradition of the Prophet Muhammad who is reported to have said that "knowledge is like the lost camel of a Muslim. Take hold of it whenever you come across it". "Our people forget", says Suhaimi, "that Islam is within us: we put the science to Islamic or un-Islamic use. Science is Islamised in the way we practise it and utilise it", he says.

Zafar L. Sawaf, Director of Centre for Industrial Research and Development, presents a similar argument. "Islam comes not with science, but after it", he says. "To what use do we put science in our societies? This is the Islamic question". Sawaf argues that science, in the Muslim world, has to be pursued for its own sake. "But we cannot allow certain results of science to destroy

our values and culture and take our societies towards excessive materialism. We must exercise discretion in the use of scientific results and in pursuing research projects we must be aware of the effect our results could have on the moral fibre of our society", he says.

For Sawaf, the use to which science is put in society must be determined by Islam. "It is a question of preserving our values and culture. That science has led industrialised societies towards an ugly materialism and an excessive consumerism, we cannot deny. We cannot let that happen in Syria", Sawaf declares. "What we need is an Islamic approach to science."

Goal-oriented science

Values and preservation of culture also figure largely in the thinking of those who take the third stand on Islamic science. This group suggests that Islamic science is more a matter of philosophy than science but at

the same time, it argues that both modern science and technology are distinctively occidental. Today, throughout the world all significant science is western both in style and method, whatever the pigmentation or creed of the scientist.

Ali Kattani, Professor of Electrical Engineering at the University of Petroleum and Minerals in Dahrán, Saudi Arabia, makes the point: "Science is intricately linked with ideology in its emphasis, scale of priorities, control and the direction of research, to such an extent that scientists have now become ideologues. Their craft is not neutral, but promotes a certain pattern of growth and development and a certain ideology."

According to Kattani the Muslim scientists are only apprentices of the western scientific establishment. "Most of the research that is going on in the Muslim world is taking our societies away from Islam", he says. It has linked the Muslim scientists scientifically and emotionally with the western establishment. "So we are

Foundation for science, technology and development

AN important decision concerning science and technology in the Muslim world was taken by the Tenth Conference of Foreign Ministers of Islamic States meeting in Fez, Morocco, during 8-12 May. The decision related to the establishment of the Islamic Foundation for Science, Technology and Development (IFSTD), a supranational body that will promote, coordinate and, some hope, supervise science and technology activities throughout the Muslim world.

IFSTD had been on the agenda of the Foreign Ministers Conference for at least five years; the project was first proposed to the Fifth Islamic Foreign Ministers Conference, held at Kuala Lumpur in June 1974. Since then several studies on IFSTD have been prepared; and for the last three years a Group of Experts has been meeting to draw-up plans for launching the Foundation and finalise the \$50 million first phase of its expansion.

The news of the establishment of the Foundation was greeted with considerable enthusiasm throughout the Muslim world. As Ariffin Suhaimi of the University of Agriculture, Serdang Selangor, Malaysia, says, "the Muslim scientists have been waiting for some considerable time for an international organisation which encourages R&D activities within an Islamic framework, and which would help solve some of the current problems of the Muslim world in particular and mankind in general. At last the Islamic Conference has responded to our needs". Many Muslim scientists see IFSTD as a key instrument in bringing the Muslim scientists together. Says Waqar Husaini of Department of Civil Engineering, King Abdul Aziz University

of Jeddah: "to promote cooperation and coordination in science and technology within the Muslim world is to strengthen the bonds of Islamic solidarity".

The greatest hopes, however, are placed on the funding function of IFSTD. Ahmad Bourani, Director of Institute de Recherche Scientifique et Technique, Tunis, sums up the feeling of many Muslim scientists. "IFSTD can easily become another Muslim organisation with a rather large bureaucracy, unless it actively seeks viable projects that are dormant because of lack of funds. Tunisian examples would be research on arid zones, water economy and olive plants. We used to get some UNESCO backing. But it seems that they have lost interest in us. Let IFSTD pick up where UNESCO left us".

The Charter of IFSTD outlines its interest: geological surveys and resources maps, energy resources, petroleum, water resources and desalination, electronics, medical research, oceanography, space research, defence technology, ecology and food and agriculture. The accent is definitely on high technology; there is conspicuous absence of alternative technologies, though solar energy gets a passing mention. A vague reference to technology assessment is made when it is stated that the Foundation will "conduct studies on the impact of utilisation of science and technology in socio-economic plans on Islamic culture and heritage".

There are, however, a number of very original goals that IFSTD hopes to pursue, the realisation of which would make a considerable contribution to science development in the Muslim

world. These include the development of "appropriate (Muslim) models of science policy" and assistance to Muslim countries to adopt them; preparation of "plans for the training of scientific manpower capable of disseminating Islamic scientific culture", and "establishment, where needed, of scientific and technological institutions in (Muslim) countries, particularly technical universities in Africa and South East Asia".

IFSTD will also study and make recommendations to Muslim countries for improving the status of scientists and technicians. In particular, special care will be given to Muslim scientists working in the West; IFSTD even proposes "to set up one or more Islamic universities for science and technology in the (developed) countries".

Science policy organisations of all Muslim countries will particularly attract IFSTD's attention. The foundation will urge them to operate within an "Islamic strategy", to promote science and technology at all levels of society, and give priority to "the creation of distinctive technologies indigenous to the Islamic world". Countries which do not have national organisations responsible for science policy and planning will be urged to establish such bodies which should be affiliated to the highest authority in the country. Where it is not possible to establish such bodies, IFSTD will undertake advisory role in science planning and policy making.

The Islamic Secretariat in Jeddah, which is also the headquarters of IFSTD, is now actively looking for a director — an "eminent scientist" — to lead it. □

working for a society that is elsewhere, in the West. Most of our work ultimately benefits the developed countries", he argues. "Because we are working within a system of science whose emphasis and priorities reflect the aspirations of an alien culture, science in the Muslim world is either in a state of struggle or in a state of continuous degeneration".

Islamic science, according to Kattani, is that science which reflects the needs and aspirations of Muslim people. "Western science places man at its centre, in the position of a demi-god. Islamic science emphasises the position of man in the universe: he can rise to the level of angels or sink to unimaginable depths, but he is responsible for all his actions, and the after-effects of his action, to a Supreme Authority. Scientific activity carried out under such a realisation is an entirely different proposition. The actual content of such a science may not be radically different. But the priorities and emphasis



Abdullah Naseef: science serves goals

will certainly be different. And so will the quality and quantity of the content of this science. And finally, the use to which this science will be put will also be different", he says.

The argument is further clarified by Raghieb El Naggar, Professor of Geology also at the University of Petroleum and Minerals. "The belief in a Creator makes Muslim scientists more conscious of their activities. It places their reason and curiosity under the authority of a Supreme Power to whom they are responsible for all their actions". He quotes the verse of the Qur'an that says: "whosoever kills a human being for other than manslaughter or corruption on Earth, it shall as if he has killed all mankind; and whosoever saves the life of one, it shall be as if he has saved the life of all mankind". "We, therefore", says Naggar, "cannot let our scientific curiosity run blind for if our activities result in a single death, even indirectly, we will be accountable for it".

Thus, Naggar argues, science in Islam must be a goal-orientated activity. "Islamic science", he says, "cannot be isolated from wisdom. Neither can it be isolated from values". The integration of wisdom and values in Islamic science, according to Naggar, is in no way a

restriction on thinking. Rather, "it gives one's thinking a direction". He rejects the notion of science for science's sake and considers the social function of science to be more important than science itself. "Our destiny as Muslims is determined not by science *per se* but by its social function", he says.

The fourth and the final stand on Islamic science also emphasises the social function of science but argues that Islamic science has its own, unique entity that differs considerably from science as it is practised and nourished today. Western science, it is argued, draws its inspiration from the Enlightenment and its assumption are those of the *Philosophes* and the rationalist world-view. Some of its underlying assumptions are those of medieval Christianity. As such, science, as we know it today, is a product of western civilization and an embodiment of its culture, ethos and values.

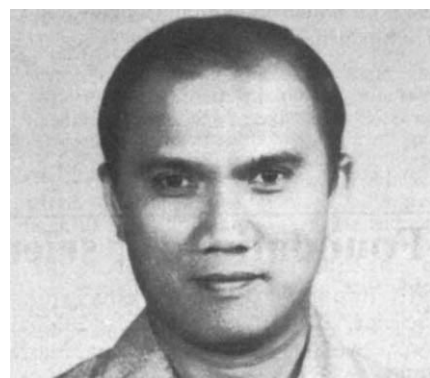
The proponents of Islamic science argue that Islamic science is based on entirely different assumptions about the relationship between man and man, man and nature, universe, time and space. Because the basic axioms of Islamic science are different from those of western science, and its method of knowing is also different, it is a science with its own identity and character.

Says Abdullah Naseef, Vice Rector of King Abdul Aziz University of Jeddah, "under Islam, science is subservient to the goals of society. The goals of an Islamic society are to increase brotherhood, reduce consumption and increase spiritual awareness. A science with these goals has to be different in nature and style from science as it is practised today. Furthermore, these goals cannot be pursued by any means. They can only be pursued by those means which are permitted by Islam. Aggressive rationalism, to give an example, is considered by Islam to be anti-human. So Islamic science does not accept the tyranny of one, supreme method. We emphasise methods in conformity with the nature of enquiry. Within these parameters, Islamic science has access to reason and experience, observation and experimentation, deduction and induction but always we turn to revelation as the supreme authority. Islamic science is the most exact of sciences without being fooled into believing that the method of experimental and theoretical sciences can lead to eternal truths."

In the post-revolution Iran, there are many scientists who hold this view. And one hears these arguments time after time. Mahdi Mumkin, Deputy Minister at the newly established Ministry of National Guidance in Iran and a theoretical architect of the Iranian revolution, holds very similar views but adds that Islamic science keeps the long-term future of society in perspective. "Muslim scientists do not seek short term expedient answers to society's needs. The long term view is an essential

element of Islamic science. The pile-up of military weapons in the US and the Soviet Union, to give an example, is totally unrelated to any concept of a viable human future", he says. Mumkin makes an even bigger claim: "the axioms of Islamic science, its concepts of man, nature, environment, and the universe, do not permit Islamic science to become part of a system of violence — and violence is defined here in very broad terms — or of a system of power and control", says Mumkins.

Waqar Husaini of the Department of Civil Engineering, King Abdul Aziz University, Jeddah, suggests that this is because "Islamic science is



Affifin Suhaimi: objective and Islamic

epistemologically and ontologically inseparable from the Islamic concept of God." He argues that under no circumstances can Islamic science pursue research "at the expense of socio-ethical indicators of spiritual development, sanctity of family life, exploitation of natural resources without due consideration or future generations, and weapons, such as nuclear, chemical and biological, which have no regard for humanity". According to Husaini, "Islamic science is a public interest science. It operates within the parameters of the Islamic concept of *al-maslaha* (loosely translated as the "common good") and promotes, preserves and develops 'five universal principles' which constitute the common good, namely — the Islamic way of life, life itself, reason, posterity and property".

Clearly Islamic science has a strong input from the ethics and morality of Islam. It is equally clear that it is a fuzzy concept that needs a great deal of clarification. For it to become acceptable to a wide cross-section of Muslim scientists, as well as western scientists, Islamic science has to prove itself both theoretically and practically. Considering the vast array of issues, from food, water, housing, transportation, medicine to health that now confront the Muslim world, there is no shortage of problems to work on. It will be worthwhile to observe if Muslim scientists can show that Islamic science has its own unique nature and style; and live up to their declared ideals. □

correspondence

Fast reactors will be problematical and expensive

SIR,—I did not reply at once to Dr Smith's letter (23 August, p630) which criticises my article (26 July, p 270) on fast reactors as I was not sure quite how to reply. It now seems to me that the best way I can deal with the issues he has raised and his list of my supposed errors — elementary, outstanding and otherwise — is to quote other authors who have made similar points, with appropriate references so that any interested readers can check for themselves. So this letter is rather long but the issues are important in view of the continuing discussion in Britain on CFR 1.

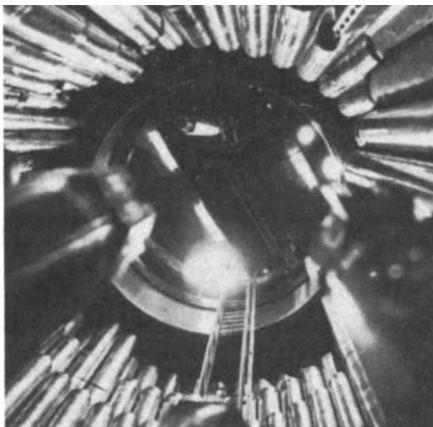
I will start with my four "outstanding errors" in physics. One is the misprint on the Doppler effect. It is remarkable that Dr Smith felt it necessary to refer to this "error" as the full version¹ of the lecture I gave at a conference in London last year has been available to him at Risley since May. My July article was simply a shortened version of the lecture, but there was no misprint in the full version.

The second "outstanding error" is my statement that a thermal reactor has less than one critical mass. If Dr Smith does not accept me as an authority on this point, perhaps he will accept Hans Bethe (Director, Theoretical Physics Division, Manhattan Project, Los Alamos 1943-46; Professor of Theoretical Physics, Cornell University since 1937; Nobel Prize for Physics 1967). Bethe² states in his testimony in support of nuclear power to the State Legislature of California in 1975: "The most important difference between a fast and a slow neutron reactor is that a fast reactor contains more than one critical mass. (By contrast the material in a light water reactor can never form a critical mass because the concentration of the fissile isotope ²³⁵U is very low.)" The point is, of course, that a thermal reactor goes critical only in the presence of a moderator whereas a critical mass of a substance is that mass (at normal density) which goes critical by itself.

The third "outstanding error" is that "thermal reactors are designed to be in their most reactive nuclear configuration". This is indeed a possible design requirement for a thermal reactor, although not for fast reactors, and is sensible design practice for commercial thermal reactors. Compare Fauske's lecture³ at the International Conference on Containment of FBRs at San Francisco in 1977 where he said "Unlike LWRs LMFRs can be very sensitive to dimensional changes or relocation of core materials since the intact LMFR core is not in its most reactive configuration". Farmer's examples (12 April, page 593) are not really relevant — *overcooling* is not a hazard for electricity-generating PWRs although it may have caused incidents in small submarine reactors.

My fourth "outstanding error" is the re-criticality scenario leading to a core-disruptive accident. A more detailed account of such accidents is given in Wilson's article⁴.

So much for the "outstanding errors". Dr Smith then claims that "these errors are important in that they convey the impression that fast reactors are difficult to control, unsafe and not well understood. On the contrary . . . Their safety has been the subject



Inside Dounreay's PFR

of intense study for very many years and is well understood." I agree that FBR safety has been studied intensely for many years; unfortunately, however, it will have to be studied intensely for many more. This is in fact the conventional view. At the 1976 meeting in Chicago on Fast Reactor Safety, spokesmen for the UK Nuclear Installations Inspectorate stated⁵ "we have indicated the seriousness with which the NII views the possibility of whole-core accidents in a thermal reactor system . . . At the present time however we believe that there are doubts about the adequacy of fault lists, about some of the physical phenomena which might play a part in an accident sequence, about structural materials behaviour, and the performance of protective systems . . . A sound programme of theoretical analysis backed up by suitable model tests is a minimum requirement." My claim that proceeding with a large fast reactor such as CFR1 with a large positive sodium void coefficient will cause even more problems is also shared by others, even by strong proponents of fast reactors such as Professor Bethe⁶. He said at Chicago⁷: "In my opinion, reactor should if possible be so designed that there is no positive void coefficient."

If we now turn to FBR economics, the argument hinges on two basic points⁸: the comparative capital costs of FBRs and other reactor systems, for example PWRs; and the future price of uranium. Keck⁹ has demonstrated in the only case study¹⁰ available that SNR-300 will have cost 5 times as much as an equivalent PWR when it is completed. Dr Smith's comments on SNR-300 are incorrect and the interested reader should consult Keck's massive thesis¹⁰ where a blow-by-blow account is given of how the increased costs were incurred. It may well be that the factor 5 will not be appropriate for the cost of CFR1 compared with a PWR system. Dr Smith claims that an estimate of this factor would be 1.5 ± 0.5 . I would suggest that it would be wise for the government and electricity board concerned to double this estimate to 3 ± 1 in line with the factor of 3 assumed¹¹ for the proposed Clinch River breeder reactor in the US. The economist Duncan Burn who has studied the British nuclear industry for many years¹² made essentially the same point at the London conference last year¹³: "I do not doubt that

following on the long development of the FBR in the UK which has culminated so far in the PFR at Dounreay we can at great cost proceed to construct one or more large 1300 MW prototypes and make a plant which will work and be safe. But it would not necessarily satisfy the economists' criteria."

Dr Smith then claims that "there is little doubt that lower fuel cycle costs will more than compensate as uranium prices increase due to pressures in the next few decades on limited supplies of uranium ore". My colleagues at the Science Policy Research Unit here are engaged in an SRC sponsored study of fuel cycle costs. Their conclusions on the future price of uranium, in line with authoritative sources in the uranium industry, are that¹⁴ "In the longer term reserve estimates are rising rapidly, demand growth is being cut back, higher grades of ore will come on stream in the 1980s and some cost-cutting technical improvements are likely. In the probable absence of an effective cartel, this will provide strong pressure towards a lowering in the long-term real price of uranium for the remainder of this century and possibly even longer."

Dr Smith ends his letter by regretting that my article "gave such an unbalanced view of fast reactor safety". I hope that your readers will prefer the opinion of a distinguished scientist in government service who wrote to me saying "what an excellent and very fair summary".

Yours faithfully,

NORMAN DOMBEY

University of Sussex, Brighton, UK.

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Affecting the effect

SIR,—While affiantly reading page 618 in *Nature* of 25 October, I found it effascinating how an effroyable use of 'effect' effectively effeebled the meaning. Effacing the 'e' and affixing an 'a' had an effacious effect on the affected sentence. The affeebled article's meaning now effulged.

Your affatuated reader,

T. W. COLE

CSIRO, Epping, New South Wales, Australia.

news and views

Anomalous ionic conductors

from Robert W. Cahn

GOOD ionic conductors such as beta-alumina, zirconia and silver oxide are the object of research in a number of laboratories on account of their potential use in high-performance batteries such as the sodium/sulphur battery. These conductors are ionically bonded crystals in which one ionic species migrates readily under the influence of an electric field; there is no electronic conduction of the metallic type.

Two recent papers in the Chinese journal *Scientia Sinica* indicate that Chinese scientists are also actively investigating at least one ionically conducting species, though curiously their interest does not seem to focus on electrical behaviour. Yang Zhen, Niu Shiwen and Cheng Yufen at the Institute of Atomic Energy of the Academia Sinica (*Scientia Sinica*, 22 (9), 1000; 1979) have studied the anomalous enhancement of neutron diffraction intensities from α -LiIO₃ resulting from an applied non-alternating electric field. The α -LiIO₃ crystals (made at the Institute of Physics of the Academy) are hexagonal and the field was applied along the *c* axis. The integrated intensity of the (0002) diffraction peak, measured by neutron diffraction, is sharply enhanced by a field: the factor of enhancement can reach 6 times, and saturates at a few kV cm⁻¹. α -LiIO₃ is polar (non-centrosymmetric) and it is thus not unexpected that equal fields of opposite polarities have effects of different magnitude on the diffracted intensity.

The applied field enhances the width of the diffracted peak as well as its height. *a.c.* fields also have an effect though this drops as the frequency is raised and disappears above $\sim 10^3$ Hz. The effect is anisotropic and (100) and (010) reflections are not influenced by a field, whatever its direction. Curiously, X-ray diffraction is very little enhanced!

Systematic experiments were done on relaxation of the diffraction enhancement at various temperatures in the range 8–71 °C; relaxation of the whole integrated intensity and of the height of different parts of the

diffraction peak were separately studied. A particularly intriguing series of experiments involved heating a crystal from ~ 100 K with a field switched on and monitoring the diffracted intensity continuously. The enhancement effect does not show up till the temperature passes 200 K, and above this same temperature an increasing ionic current flows through the crystal.

This paper is accompanied by a second, from the Academy's Institute of Physics (Gu Benyeen, Xu Zhengyi, Zhao Shifu and Zhang Andong, *Scientia Sinica* 22 (9), 1010; 1979). Here a *d.c.* field applied to α -LiIO₃ crystals is found to enhance the diffraction of light. This fact had been previously reported, and the effect depends on field polarity, field strength and temperature in much the same way as does neutron diffraction. The present paper works out the mathematical implications of the hypothesis that the *d.c.* field drives mobile impurity ions along the *c* axis until they come to rest along a series of layer-like defects (stacking faults?) lying parallel to the six pyramidal planes of the form {101}. The hypothesis is confirmed both by comparing the anisotropic diffraction patterns recorded in the presence of a field with calculated patterns, and by micrographs of a slab cut in such an orientation as to show a single family of defects edge-on.

The defect-piling hypothesis can presumably explain the neutron diffraction anomaly, though it is not altogether clear why that effect is restricted to the (0002) reflections, in view of the fact that the defects are not perpendicular to the *c* axis.

The literature cited in both papers indicates that the two laboratories have been systematically studying α -LiIO₃, at least since 1972. The initial motive may have been, at least in part, interest in the applications of this crystal in non-linear optical devices (see for example Sailor *Phys. Stat. Sol. (a)* 4, K173; 1971), but the near success in confirming the origin of the various anomalies deserves attention from the various groups elsewhere studying ionic conductors.

Several of these conductors in fact contain lithium, and intensive research on several such crystals has been done in

recent years, especially by a team at the Max-Planck-Institut für Festkörperforschung in Stuttgart. Some species show uniaxial preferred ionic conductivity, like α -LiIO₃; thus β -eucryptite, LiAlSiO₄, has a hexagonal quartz-like structure with lithium ions arranged in channels parallel to the *c* axis, and conductivity $\parallel c$ is about 10³ times higher than $\perp c$ (v. Alpen, Schulz & Talat *Solid State Comm.* 23, 911; 1977; *Electrochim. Acta* 22, 805; 1977). It would be most interesting to test whether α -eucryptite responds anomalously to *d.c.* fields in a way similar to α -LiIO₃, another uniaxial conductor.

Another Li-bearing ionic conductor, Li₃N, which has a layer structure of Li₂N and pure Li layers, has been extensively grown and investigated in Stuttgart, and a detailed survey of its properties has been published (Rabenau *Festkörperprobleme* 18, 77; 1978). X-ray structural studies of quite remarkable precision (Schulz & Schwarz *Acta Cryst.* A34, 999; 1978; Schulz & Thiemann *ibid.*, in the press) have shown that at room temperature the Li₂N layers have about 1% of Li⁺ vacancies whereas the interleaving pure Li⁺ layers are fully occupied. At 400 °C, the vacancy concentration in the Li₂N layers goes up to 4%. On this basis, the considerably higher ionic conductivity in the plane of the layers compared with that at right angles can be interpreted. This crystal species, therefore, has two-dimensional rather than one-dimensional conductivity, and if Li₃N shows any behaviour akin to α -LiIO₃, it may well obey quite different crystallographic laws.

The Stuttgart experience suggests that the best way to understand anomalous behaviour in ionic conductors is to combine a number of quite distinct experimental techniques focused on the same crystal species, preferably including structural analysis at a very high level of accuracy. This seems also to be the strategy that the Chinese are following in their study of α -LiIO₃.

Yang's interesting experiments on frozen impurity migration at low temperatures put one in mind of the little-known technique called Ionic Thermo Currents (ITC). This method, formally

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analogous to thermoluminescence analysis, involves cooling an ionic crystal in a field, switching off the field and then reheating the crystal connected to an ultra-sensitive electrometer which detects the microcurrent resulting from the randomisation of the aligned vacancy-impurity electric dipoles (Bucci & Fieschi *Phys. Rev. Lett.* 12, 16; 1964; Strutt, Weightman & Lilley *J. Phys. E. Sci. Instr.* 9, 683; 1976). This technique could perhaps be applied with advantage to α -LiIO₃, to

monitor the microcurrents resulting from the randomisation of segregated ionic impurities.

It would be fascinating to know why X-ray diffraction intensities from α -LiIO₃ are affected by electric fields so much less strongly than are neutron diffraction intensities. Perhaps the active impurities will need to be identified and their atomic scattering factors for X rays and neutrons identified, before sense can be made of this anomaly within an anomaly. □

Standards for microwave radiation

from R. P. Blackwell

THERE has been growing concern in recent years over the possible biological consequences of exposure to microwave and radio frequency radiation, partly stimulated by the thousand-fold difference between the maximum permissible exposure standards current in Western and Eastern Europe. The much stricter Eastern standards have raised questions of safety in the West, but a realistic appraisal of the basis for this difference is hard to achieve.

Microwaves and radio frequency radiation are absorbed to a greater or lesser extent by biological materials, the energy appearing as heat; the predominant mechanism being dipole relaxation of water molecules. The photon energies of this radiation lie in the range 10^{-3} to 10^{-7} eV; orders of magnitude below the activation energies of molecular interactions (Cleary, *Health Phys.* 25, 387; 1973). The generally accepted view is that the biological changes which are observed, such as cataract formation in the eye, are a result of heat production, although the view that non-thermal effects are important at low levels of exposure is held by some, particularly in Eastern Europe.

The Western standard of 10 mW cm⁻², which is considered to be the maximum safe exposure, was derived from thermal considerations. Because the body effectively deals with the transport of quantities of heat greatly in excess of the basal metabolic rate of 1 W kg⁻¹, whole body exposure at a power density which results in the dissipation of about 1 W kg⁻¹ of heat is considered to be safe. The Soviet exposure standard seems to be the result of a different philosophy; their industrial safety standards often represent an ideal rather than a practical limit. They assume that any detectable biological change constitutes a hazard and consequently their exposure standard of 10 μ W cm⁻² was reached by adding safety factors to a power density at which no change has been observed.

Many Soviet papers report biological effects at power densities which do not seem capable of producing any thermal change, and are therefore often ascribed to

'athermal' effects. An appraisal of much of this research is difficult. Many reports which are quoted in reviews are not available in translation; most of those which have been translated are difficult to follow. Of the reviews available, that by Baranski and Czerski (*Biological Effects of Microwaves*, Dowden, Hutchinson & Ross, 1973) is among the most recent and comprehensive. Many of the reports of experiments performed by Soviet workers have been criticised, particularly in terms of dosimetry and experimental detail. For example many of the apparent effects on the nervous system detected by electroencephalography (summarised by Baranski and Czerski) need careful interpretation. The presence of recording electrodes during irradiation and simultaneous recording makes it impossible to rule out artefacts due to thermal and electrical stimulation by the electrodes themselves.

A large proportion of reports from Eastern Europe concern behavioural, electrophysiological and strongly subjective changes of a generalised nature, which are notoriously difficult to quantify. Fatigue, memory loss and loss of concentration have been reported. Such reports include, for example, surveys of the health of people exposed to low levels of microwave radiation in the course of their work. The body of evidence produced in the West is not in accord with many of those findings.

A recent publication of the National Research Council of Canada (*The Biological Effects of Radiofrequency and Microwave Radiation*, Assenheim *et al.*, NRCC 16448; 1979) provides an objective, well referenced synthesis of the important topics in this field. What is particularly useful is the attention paid to explaining the relevant backgrounds in physics and biology, and the philosophy behind the setting of exposure standards. Research in this field is complicated by the experimental problems of exposure and dose measurement. The interaction of electromagnetic radiation and biological tissues depends on several variables, including the wavelength of the radiation and the size and electrical properties of the tissues. Diffraction and reflection may

occur at tissue interfaces, which may lead to focusing and resonance, with the consequent uneven distribution of heat, and formation of 'hot spots'. Assenheim *et al.* provide a comprehensive survey of the important topics in this area, dealing with both the prediction of absorption patterns and the techniques for temperature measurement required.

There is now sufficient material available, for example the *R. F. Dosimetry Handbook* of Durney *et al.* (Report SAM-TR-78-22, USAF School of Aerospace Medicine, Texas), to enable reasonable estimates to be made of the power distribution in a given situation. Thus it is now possible to extrapolate experimental data on biological effects to man, and to predict with a reasonable degree of accuracy the pattern of power deposition in man in most exposure situations, and the resultant biological effects, if any. Such information is necessary for the design and implementation of realistic, practical safety standards.

Many questions remain unanswered. Perhaps one of the most interesting is posed by the work of Bawin *et al.* (*Ann. N. Y. Acad. Sci.* 247, 74; 1975). They report that changes in the calcium ion efflux of chick and cat brain tissue *in vitro* can be modified by exposure to amplitude modulated VHF fields of 1 mW cm⁻². Unmodulated fields do not produce an effect; however electrostatic fields alternating at the modulation frequency (between about 6 and 30 Hz) do. These exposure values are somewhat lower than might be expected. This could have implications for effects on the central nervous system, if a similar process occurs *in vivo*.

There remain many problems associated with experimental work, particularly in terms of physiological measurement in a microwave field. Such measurements rely on transducers which normally contain some metallic components, and these may distort the field and lead to unpredictable power deposition patterns. It is possible that some of the confusion over low level effects is a result of this. For example, a metal coaxial recording electrode, implanted in cat cortex, can cause a complete shift in the pattern of power absorption, and an increase of two orders of magnitude in power deposition near the electrode tip.

Although the controversy over thermal versus athermal effects has not been completely resolved, it has had the effect of stimulating a re-appraisal of exposure standards throughout the Western world. One major development which any revised standard must take into account is the resonance effects which occur in man in the low VHF region. It is likely that more attention will also be paid to considering the amount of power that temperature-sensitive organs, or those with a poor blood supply — for example the lens of the eye and the testis — can safely dissipate. □

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Do nuclei have a neutron skin?

from John Edgington

EXACTLY 50 years ago the systematics of alpha-decay led Rutherford to the conclusion that the radius of the uranium nucleus must be less than 10 fm (10^{-14} m). An explanation for this incredibly small value, and an understanding of how and why it varied between nuclides, was a prime objective of the liquid-drop model. This viewed the nucleus as a close packed, incompressible array of neutrons and protons; the observed constancy of the binding energy per nucleon was seen as a consequence of the short range of the nuclear force and the resultant bonding between nearest neighbours only. The effect of this 'saturation' of bonds is that the nuclear radius increases as the cube root of the nucleon number, in agreement with earlier deductions from alpha-particle scattering. The more recent, and very extensive, measurements of electron scattering distributions have added flesh to this skeleton, and the distribution of electric charge within nuclei is now known quite accurately.

The distribution so revealed is remarkably model-independent. It is characterised by an almost constant central density, a rapid decline within one or two fm to a low value and an exponential decay thereafter. These features can be reproduced by any of a wide variety of shell models which, with their assumption of quasi-independent nucleon motion within some effective potential, have replaced the liquid drop model in structure calculations. The charge distributions themselves are thus too blunt instruments for the task of discriminating between the various models.

What then of the matter distribution, that is, the joint density of protons and neutrons? There is evidence, at least to first order in the electromagnetic corrections, that the strong forces between nucleons are charge independent, that is, indifferent to whether the particle is a proton or a neutron. One therefore expects protons and neutrons to have similar, if not identical, density distributions in nuclei. In terms of the single parameter which often summarises experimental results, their rms radii, $\langle r_p^2 \rangle^{1/2}$ and $\langle r_n^2 \rangle^{1/2}$ would be equal. Indeed, in a study of isobaric analogue states, Nolen and Schiffer showed 10 years ago that this equality holds quite well for a variety of medium to heavy nuclei (*A. Rev. Nuclear Sci.* **19**, 471; 1969).

Many subtle effects, however, conspire to produce this near-equality. For example, the destabilising effect of Coulomb repulsion causes all but a few light nuclei to contain fewer protons than neutrons; despite this, the excess neutrons do not contribute very much to the total

strength of the pairwise n-n bonds. This follows from Pauli's exclusion principle which limits the interactions between identical particles. The total p-n bonding strength is not so limited. The presence of excess neutrons thus has little effect on the effective potential in which the neutrons move, although strengthening that experienced by the protons. Thus the protons are more tightly bound, and confined to a smaller radius. Conversely the Coulomb repulsion acting on the outer protons will tend to expel their wave functions and thus increase the protons' rms radius. The interplay of these competing effects, and others of lesser magnitude, is imperfectly understood, and in addition there is conflicting experimental evidence for a neutron-rich region, or 'skin', at large radii.

For example, isotope shifts in atomic spectra indicate only small differences, of varying sign, between the charge radii whereas similar shifts in the spectra of muonic atoms correspond to somewhat larger differences, the charge radius generally becoming greater as neutrons are added in heavier isotopes. The whole problem has been addressed afresh by the meson factories with their intense beams of muons and pions. For example, isotope shifts in pionic atoms have been measured at LAMPF, Los Alamos, and a sensitivity to radii changes of 0.02 fm is claimed. Unfortunately, in many of these experiments, the extraction of radii differences from the raw data is an opaque and model-dependent process, and theoretical uncertainties swamp the experimental ones.

A new method of measuring neutron radii has been reported from TRIUMF, Vancouver, which, it is claimed, is not only very sensitive to different values of $\langle r_n^2 \rangle^{1/2}$ but is also virtually model-independent. In contrast to other radius determinations, this measures the neutron radius directly, not a difference between the charge and matter radii. R.R. Johnson *et al.* (*Phys. Rev. Lett.* **43**, 844; 1979) have scattered low energy π^- beams from pairs of isotopes, and measured the ratio of their differential cross sections. This ratio, it turns out, is sensitive to differences between the neutron radii of the two isotopes, almost regardless of what proton radius is assumed. The basic cause of this sensitivity is that the amplitude for π^- -n scattering is much larger than that for π^- -p at low energies, but the measurements at TRIUMF have, as a bonus, an additional enhancement arising as follows. The difference between π^- -n and π^- -p scattering arises from an interference between s and p-wave π^- -nucleon amplitudes, which has its maximum effect at extreme forward and backward scattering angles. Now in π^- -nucleus scattering a Coulomb-nuclear

interference term is of course present, and Johnson *et al.* had already shown (*Phys. Lett.* **75B**, 560; 1978) that at very forward angles this interference is constructive for π^- -scattering, but destructive for π^+ . Thus any variations in neutron density distributions should show up with enhanced sensitivity in π^- -scattering compared with π^+ . Indeed a recent measurement of π^+ - ^6Li scattering at 50 MeV (*Phys. Rev.* **C18**, 2316; 1978) saw no such effects.

Johnson *et al.* scattered π^- beams of 30 and 50 MeV from $^{12,13}\text{C}$ and $^{16,18}\text{O}$, and their results indicate that the heavier isotope has the greater neutron radius, by 0.04 fm and 0.21 fm in the two cases. Quoted errors of ± 0.03 fm in each case include, for once, an estimate of the sensitivity to variations between the different models used in the calculations. The relatively small change between carbon isotopes is of particular interest.

Some other techniques for measuring neutron radii, such as studying the absorption of K^- mesons, find a gross neutron excess in the nuclear surface. In comparing the various methods one must bear in mind that the radial distances being sampled are very different; kaonic and antiprotonic atoms are probes of the farthest reaches of the nuclear surface, whereas the experiment of Johnson *et al.* is investigating the region of rapidly rising density close to the rms radius. It is to just this region that the various nuclear models principally address themselves, and this new evidence, the first measurement of $\langle r_n^2 \rangle^{1/2}$ independently of $\langle r_p^2 \rangle^{1/2}$, has an importance that is more than circumstantial. □

Next in succession

from Peter D. Moore

MUCH current research on the process of succession in forests concentrates on the probability of a given species being replaced by an alternative species within a number of generations. This can lead to the development of a model describing a replacement series (see for example, Horn in *Theoretical Ecology* (ed. R.M. May) 187 Blackwell, Oxford, 1976). Certain non-linearities are quite frequently encountered, such as the persistence of populations from early in the sequence as a result of regular destruction of the forest, either by natural or human agencies. There are occasions on record, however, where such persistence of what one would normally consider an early successional tree species, can occur even in the absence of regular forest disturbance. The tree may even form the dominant component of an

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apparently stable community.

Perhaps the most familiar example in Europe is the birch forest of the north-western extremities of the continent, and which is best developed in northern Norway and Finland. This forest (described floristically by Hamet-Ahti, (*Ann. Bot. Soc. Vanamo* 34 (4); 1963) is an open scrub of birch trees 2–12 m in height, with no other tree species present, except scattered pine (*Pinus sylvestris*) in the more southern regions. Taxonomically there is some degree of confusion since at least two subspecies of *Betula pubescens* (ssp. *pubescens* and ssp. *tortuosa*, *sensu Flora Europaea* (ed. T.G. Tutin *et al.*) Cambridge, 1974) are present, but these are trees which would be replaced by others at lower latitudes.

It is of interest to note that arctic and subarctic forests dominated by birch also occur in certain subalpine situations, for example in the Carpathians, and in parts of the Eurasian steppes, but that they tend to be mainly associated with oceanic areas, such as Kamchatka in Pacific north-east Asia. In north America, the birches are accompanied by coniferous trees (such as *Abies*, *Picea* and *Larix*) and form pure stands only under disturbed conditions. The birch forests of northern Fennoscandia, by contrast, seem to have been stable over a considerable period. Fossil pollen data from these areas show an invasion of pine into the region between 8000 and 6000 years ago (Birks & Saarnisto *Boreas* 4, 77; 1975) but the pine pollen levels attained by 4000 years ago are no higher than those of the present day (Hicks *New Phytol.* 78, 715; 1977; Prentice *Boreas* 7, 131; 1977). It seems therefore that the northern birchwoods have not developed in response to any human disturbance. The same applies to the birch woods of the northern part of the Isle of Skye in Scotland (Vasari & Vasari *Acta — Bot. Fenn.* 80, 1; 1968).

As noted by Walter (*Vegetation of the Earth*, Springer-Verlag, New York, 1973) the coniferous trees of lower latitudes may be held back by a failure in seed set or by desiccation as a consequence of frost and wind. Such species as *Pinus sylvestris* extend into the birch forest zone along the sheltered valleys. Regeneration of birch, which is a light-demanding tree, probably depends on the death of adults, thus maintaining an open, light canopy forest.

Birch forests of this kind are not found in the southern hemisphere, although deciduous *Nothofagus* species which form woods in southern Chile may be considered an ecological equivalent (Godley *Proc. R. Soc. Lond B* 152, 457; 1960). Recently an analogous vegetation type has been described from the Galapagos by Hamman (*Bot. J. Linn. Soc.* 78, 67; 1979). Here an endemic woody composite, *Scalesia pedunculata* dominates the humid forests of Santa Cruz Island. The growth and population dynamics of this species have been investigated by means of permanent

quadrats established in 1970 and recorded on three occasions since then. By this means the survival and growth characteristics of individual trees have been measured.

Mortality among *Scalesia* seedlings is high in the early years (only 40% survival after the first 2 years) and then decreases (2% survival after 7 years). Initial growth rates are very high, the trees reaching 7–8 m in 3–6 years. Height stabilises at about 10–12 m and girth increase becomes pronounced after about 5 years, coinciding with the onset of flowering. Few individuals survive beyond 10 years.

Many of these features such as short life span, rapid early height increase, and early flowering are characteristics normally associated with an early successional forest species. *Scalesia* persists and dominates the vegetation by means of a high seed production and a rapid population turnover. Although there is some disturbance of the forest by grazing animals, particularly giant tortoises, the *Scalesia* population does not seem to depend on this for

survival. Vegetation zones on the Galapagos generally seem to be climatically determined (Hamman *Biotropica* 11, 101; 1979) and the post-glacial climatic history of the Galapagos has been essentially stable as reflected by the vegetational history (Colinvaux & Schofield *J. Ecol.* 64, 989; 1976). The *Scalesia* woodland should therefore be regarded as climax, in the sense that no further succession should be expected.

In both of the cases quoted, the Fennoscandian birchwoods and the Galapagos *Scalesia* forest, an over-riding biogeographical factor has restricted the invasion or success of those tree species which could be defined as late successional (or 'persistent multilayer' in the sense of Horn, *The Adaptive Geometry of Trees*, Princeton University Press; 1971). In the former case the restrictive factor is climate and in the latter it is geographical isolation. These simplified exceptions to the normal course of successional processes would repay more detailed investigation regarding regeneration and survivorship.

Inside the Earth

from David T. Rickard

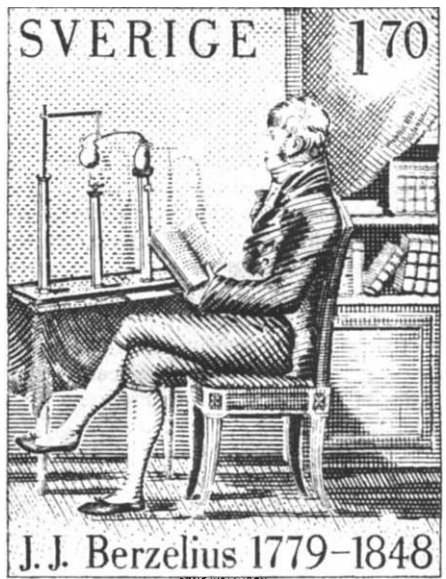
JÖNS Jacob Berzelius was born 200 years ago in the village of Väversunda in Sweden, and was to become one of Sweden's greatest, but least appreciated, scientists. Linnaeus, who had died the year before, represented the culmination of descriptive science; Berzelius' birth ushered in the analytical age.

One of Berzelius' major scientific contributions was his chemical system of mineralogy. Previously, minerals had been classified on the basis of form and colour in the same way as Linnaeus had classified plants. Berzelius chemically analysed minerals and classified them according to their compositions.

This introduction of chemistry into geological science was remembered by the Swedish Royal Academy of Sciences in their choice of a theme for a Nobel Symposium to commemorate the Bicentenary of Berzelius' birth. The Academy invited twenty-five distinguished scientists to discuss the chemistry and geochemistry of solutions at high temperatures and pressures.*

This subject is very much in the spirit of Berzelius. It represents an area of endeavour which only the most careful theoretical and experimental chemistry can hope to penetrate. It also reflects the conditions under which most of our planet exists.

The zone on the Earth where temperatures around 25 °C and pressures of about 1 atmosphere, or bar, predominate is



represented by a thin surface sheath about 12 km thick. Because we happen to inhabit this sheath our knowledge of inorganic and physical chemistry under these conditions is relatively good. However, the remaining 6,371 km down to the centre of the Earth are characterised by considerably higher pressures and temperatures. The PT (pressure-temperature) conditions in the first 250 km are illustrated in Fig. 1. It is in this high PT zone that the bulk of the Earth's rocks and ores are formed and where the major material transfer processes occur.

In contrast to 25 °C and 1 bar, our knowledge of inorganic and physical chemistry in the rest of the PT spectrum is

*The 48th Nobel Symposium was held at Bofors, Karlskoga, Sweden on 17–21 September, 1979. The Symposium proceedings are expected to be published in 1980. The symposium was organised by David T. Rickard and F.E. Wickman of the Geological Institute, Stockholm.

extremely limited. The symposium was therefore not a summary of achievements in a well-established and well-researched field. Rather it was a delineation of our areas of ignorance and a discussion of various methods of tackling the problems.

Take the best known fluid, water, as an example. S.D. Hamann (CSIRO, Melbourne) noted that our present knowledge of the PVT (pressure-volume-temperature) behaviour of water is known quite accurately up to 100 °C and 1 kbar, less accurately to 1,000 °C and 10 kbar, and progressively more uncertainly above these limits. The highest temperature reached by conventional techniques is 1,200 °C (at 4 kbar) and the highest pressure is 34 kbar (at 175 °C). Shock-wave PVT data, which are less reliable, have been obtained up to 400 kbar and 3,100 °C but are very uncertain above 30 kbar. The actual nature of water under these extended conditions is not well known. E.U. Franck (Karlsruhe University) suggested that treating it as $\text{H}_3\text{O}^+\text{OH}^-$ was a good approximation; that is, as hydronium hydroxide, very similar to fused sodium hydroxide.

The data are even more limited for systems which contain other components in addition to water. The best-known system, $\text{H}_2\text{O}-\text{NaCl}$, has only been extended this year to 400 °C, 4 kbar and 25 weight percent salt. The few other systems that have been studied are known over a much more limited range of conditions.

Apart from the mechanical problem of confining solutions at high pressures and temperatures within a limited space, the solutions of interest are often highly corrosive. H.L. Barnes (Pennsylvania State University) pointed out that a major problem concerning geochemical experimentation was the relatively long reaction times often needed to reach equilibrium. Materials have to withstand temperatures of several hundred degrees and pressures in the kilobar range for up to one month. It is not without reason that these experimental systems are called bombs.

Materials in themselves are not the only problems facing the experimentalists at high temperatures and pressures. Experimental apparatus must be constructed so that measurements can actually be made. That is, it is not only necessary to confine the reactants but also to have access to them. Particular interest was engendered by spectroscopic methods of measurements of the chemical properties of solutions up to 5 kbar and 500 °C by M. Buback (Karlsruhe University). These techniques involve constructing a bomb with a window of a transparent, chemically resistant material, such as sapphire. Spectra of the reactant solution may be examined. Infrared adsorption spectra are commonly observed, but Buback also

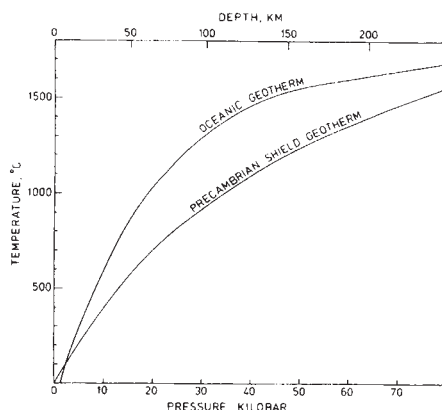


Fig. 1 Typical temperature and pressure variations with depth (geotherms) in the Earth for an oceanic and ancient Precambrian situation (from Wickman & Rickard, Chemistry and geochemistry of solutions at high temperature and pressure: an introduction to the symposium).

described the advantages and potential of laser Raman spectroscopy for these types of systems.

Even if experiments could be performed simply and quickly, the variety of solution compositions occurring in nature is so great that centuries of labour would be required to complete the work. Furthermore, it is not, at least at present, possible to examine experimentally the chemical properties of many systems under the extreme conditions that occur in the Earth. It is obvious that a major goal of high PT research is the construction of reliable methods for the theoretical prediction of the chemistry of solutions under extended conditions.

H.C. Helgeson and K.S. Pitzer, both from the University of California, Berkeley, presented alternative approaches to this problem. Helgeson discussed the 'ion association' approach, which provides specifically for ions combining together as a function of pressure and temperature. He argued that we know that solutions at high temperatures and low pressures contain a large proportion of combined or associated ions and that therefore the ion association model is more realistic.

The 'virial coefficient' approach used by Pitzer treats the solutions as though they contained only separate ions, and divergences from this ideal behaviour are accounted for in the coefficients of a single equation. Pitzer argued that the concentration of associated ions is not always unambiguously determinable and that there is essentially a continuous distribution of populations of ions at varying distances from one another.

However, these different approaches should not be regarded as competitive, but rather as complementary. For example, H. Eugster (Johns Hopkins University) showed, in a contribution to the discussion of Pitzer's paper, that use of the virial coefficient approach had solved for the first time a long-standing problem in physical chemistry — the theoretical prediction of salt precipitation during the

evaporation of seawater. No other approach had resolved this problem which has been known since van't Hoff's work at the beginning of this century. In comparison, application of the ion association model has already given much information about the nature of geological processes under extreme physical conditions — information which is not available experimentally at present nor is likely to be for some time to come.

The application of theoretical and experimental high PT solution chemistry to geological and technological problems was a recurrent theme of the symposium. On the geological side delegates discussed the formation of igneous rocks, the origin of ore deposits and the response of rocks to solutions at high temperatures and pressures. Geothermal energy was a topic of some considerable interest and various aspects were reviewed from source to power station chemistry. T. Seward (DSIR, Petone, New Zealand) combined several themes in his poignant tale of drilling for hot water and finding a source that deposited gold in large quantities. His chemical studies were able to show how the gold was transported in these hot solutions, combined with sulphur-containing ions.

One of the prime purposes of the 48th Nobel Symposium was to initiate a dialogue between chemists and geologists working in the same field, often unaware of each other's contributions. In this the meeting succeeded and several firm suggestions were made for future cooperation and for future meetings on this theme. Berzelius would have been happy! □

Erratum

In the article 'Dissecting the red cell membrane skeleton' (*News and Views* 281, 426; 1979) the sentence beginning on page 427, third column, four lines from the bottom, should read "... This suggests that the lower molecular weight forms are degradation products of the parent band 2.1 molecule." On page 429, first column, the two lines following line 13 were inadvertently omitted. The complete sentence should read "This is probably the end of the molecule where actin and band 4.1 bind since interactions between actin and crude spectrin preparations are phosphorylation sensitive (Pinder *et al.* *Nature* 270, 752; 1977). Unfortunately, so far at least, it has not been possible to recapture the phosphorylation dependence in more purified systems (Brenner & Korn *op. cit.*; Cohen & Branton *op. cit.*). On page 429, line 9 of the second column is incorrect: the complete sentence should read "This implies that spectrin has evolutionarily critical functions and, since its only known functions relate to the membrane skeleton, that the skeleton is an evolutionarily critical structure".

Interferons and cellular regulation

from Mike Clemens

INTERFERON is best-known for its antiviral activity, but the principal message of a recent meeting on 'The Regulatory Effects of Interferon'* was that the multiplicity of other biological effects attributed to it are holding up now that pure interferons from several sources are available and have been tested.

Interferon of very high specific biological activity has been prepared after induction of its synthesis in either human fibroblasts (J. Heine & A. Billiau, University of Leuven; E. Knight, E.I. du Pont de Nemours & Co.) human lymphoblastoid cells (K. Zoon, National Institutes of Health, Bethesda) or mouse Ehrlich ascites tumour cells (P. Lengyel, Yale University). Convincing evidence for homogeneity of the purified material was provided by amino acid sequencing studies conducted by M.W. Hunkapiller (California Institute of Technology). He described several technical improvements which have allowed identification of the first 20 N-terminal amino acids from less than 1 µg of interferons from each of the above sources. Interestingly, the human lymphoblastoid and fibroblast interferons show no sequence homology in this region (at either the protein or possible DNA codon level), whereas one species of mouse interferon does have some sequence similarities with human fibroblast interferon. Evidence was presented by D.C. Burke (University of Warwick) for the presence of the gene for human fibroblast interferon on the short arm of chromosome 9, rather than on chromosome 5 as has been previously suggested. As yet, however, no information is available concerning the structure of the interferon gene and reports on progress towards the possible cloning of the gene were conspicuous by their absence.

A great deal of information was presented on the effects of the various interferons on a wide range of cell types. More knowledge has been accumulated about type II or 'immune' interferon, produced principally by T lymphocytes in response to mitogenic or antigenic stimulation. Both S. Baron (University of Texas, Galveston) and E. Falcoff (Institut Curie, Paris) reported that it is antigenically distinct from virus-induced (type I) interferons. Many of its biological effects, including the induction of new enzyme activities in mouse L cells, are similar to those of type I interferon, but its ability to inhibit the growth of tumour cells is relatively higher.

The inhibitory effects of interferon on cell multiplication are also of particular interest. A.A. Creasey (Stanford University) suggested that, in some human melanoma

cell lines, interferon may prevent a metabolic event required for commitment to a new round of DNA synthesis. The nature of this event and the mechanism by which it is modulated by interferon remain unknown. However, the series of 2'-5'-linked adenylylated oligonucleotides, synthesised by an interferon-induced enzyme in several cell types, has been implicated in this regulatory system. Inhibition of thymidine incorporation into DNA by the 2'-5' oligo(A) series was reported in L cells (A.G. Hovanessian, Institut Pasteur, Paris), concanavalin A-stimulated lymphocytes (A. Kimchi, Weizmann Institute, Israel) and human lymphoblastoid cells (I.M. Kerr, National Institute for Medical Research). Whether these effects are consequences of an earlier inhibition of protein synthesis remains unclear. Techniques for *in vitro* screening of the responsiveness of human ovarian carcinoma cells from patients for possible growth inhibitory effects of interferon were described by L.B. Epstein (University of California, San Francisco).

It is now clear that the interferons also have a wide range of effects on cells of the immune system. Those effects which may be particularly important for the anti-tumour actions of interferons include activation of natural killer cells (B.R. Bloom, Albert Einstein College, New York; G. Trinchieri, Wistar Institute, Philadelphia; R.B. Herberman, National Institutes of Health) and macrophages (S.I. Hamburg, New York University; M.A. Chirigos, National Cancer Institute). Tumour cells stimulate normal lymphocytes to synthesise interferon *in vitro*, and this has obvious implications for the control of tumour growth in the intact animal. A comforting conclusion which can be drawn from much of this recent work is that the various biological effects of earlier crude interferon preparations can also be elicited using the material purified to homogeneity (J. De Maeyer-Guignard, Institut Curie, Orsay; S. Pestka, Roche Institute, Nutley). Interferon has thus acquired a respectability which should stimulate further detailed molecular and cell biological analysis of its modes of action.

The recognition that interferon has a wide spectrum of hormone-like actions has also led to the realisation that it may exert harmful effects under certain circumstances. These may include some of the symptoms associated with certain autoimmune diseases, since a high proportion of patients with systemic lupus erythematosus or rheumatoid arthritis have elevated serum interferon levels (J.J. Hooks, National Institutes of Health). I. Gresser (IRSC, Villejuif) described the

deleterious consequences of giving large amounts of interferon to new-born mice and summarised his view of these effects: "Interferon is a good thing. Too much of a good thing is sometimes a bad thing".

There have also been significant developments in understanding the anti-viral effects of interferon. There is now a considerable amount of information on the involvement of 2'-5' oligo(A) in the regulation of protein synthesis in interferon-treated cells. The interferon-induced enzyme responsible for synthesis of this series of oligonucleotides in the presence of double-stranded RNA (dsRNA) has been purified to homogeneity in the laboratory of P. Lengyel (Yale University). This group has also extensively purified both the endonuclease which is activated by 2'-5' oligo(A) and the protein kinase involved in inhibition of polypeptide chain initiation after interferon treatment. B.R.G. Williams and I.M. Kerr (National Institute for Medical Research, London) presented evidence that intact interferon-treated, EMC virus-infected cells synthesise 2'-5' oligo(A) and that the oligonucleotide will prevent virus replication when added to permeabilised infected cells. Thus the model which implicated 2'-5' oligo(A) in the antiviral actions of interferon, which was originally derived from studies with cell-free systems, has been confirmed in these whole cell experiments. L.A. Ball (University of Wisconsin), who has also extensively purified 2'-5' oligo(A) synthetase from interferon-treated chick cells, showed that the enzyme elongates the oligonucleotides in the 2'-5' direction non-processively, and will also add AMP residues to other small molecules such as NAD and ADP-ribose. C. Baglioni (SUNY, Albany, New York) described the structural requirements for dsRNA for activation of both the 2'-5' oligo(A) synthetase and the protein kinase. A minimum of 60 base-pairs of dsRNA is necessary and mismatching of bases lowers the efficiency of activation. It is not yet clear whether 2'-5' oligo(A) can inhibit viral protein synthesis selectively *in vivo*, for example by being synthesised and acting only in the vicinity of dsRNA-containing viral replication complexes, as suggested by Baglioni. In the case of Mengo virus-infected L cells host cell protein synthesis is also shut off, but later recovers in the interferon-treated cells (R. Falcoff, Institut Curie, Paris), perhaps because the 2'-5' oligo(A) is subsequently degraded by a cellular enzyme.

A more subtle way in which interferon inhibits the replication of tumour viruses was described by both P.M. Pitha (Johns Hopkins University, Baltimore) and R.M. Friedman (National Institutes of Health). Several steps in the maturation of these viruses are impaired in interferon-treated

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*Held by the New York Academy of Sciences on 23-26 October.

cells and the particles which are released show reduced infectivity. The latter phenomenon may be due to defective glycosylation of a virion protein. Interestingly, Friedman also reported that vesicular stomatitis virus produced by interferon-treated L cells is non-infectious, is deficient in G (glycoprotein) and M (matrix) protein content and appears abnormal in the electron microscope. □

Multiplying growth factors

from Denis Monard

MORE knowledge about the events regulating the initiation of DNA synthesis will certainly shed more light on the phenomena regulating the differentiation of animal cells which remains one of the main challenges of modern biology. Such basic knowledge should also contribute to understanding better how and why a tumour cell differs from a normal cell.

Opening a recent discussion* on this topic R.W. Holley (Salk Institute, San Diego) re-emphasised that all growth is regulated by a multiplicity of complex extra and intracellular events. The discovery of proteins which can trigger cell growth, such as migration factor, epidermal growth factor (EGF), fibroblast growth factor (FGF) and platelet growth factor has revealed that extracellular macromolecules have an important role as regulators of the cell cycle, and the number of such factors known is steadily increasing.

Platelets have provided several new growth factors. The stimulation of cell growth by three polypeptides released by platelets has been reported (D. Paul, Temple University, Philadelphia). All three polypeptides cross-react immunologically and chromatograph under the same conditions in heparin Sepharose. They differ, however, in their molecular weight. One of them, a basic protein, is displaced by β -thyroglobulin or EGF. But, in contrast to growth stimulation by EGF, its biological activity is impaired by heparin. Another growth factor has been obtained from platelet extracts (W.J. Pledger, University of North Carolina, Chapel Hill) but analogies with the other growth factors released by platelets are premature.

Virus-transformed BHK fibroblasts also produce and release two proteins which stimulate the migration and DNA synthesis of normal fibroblasts (P. Leuthard, Friedrich Miescher-Institut, Basel).

A cell line with an especially large number of EGF receptors has been used to

purify a sarcoma growth factor (SGF). After being fixed with formalin, these cells still bind the growth factor released by cells transformed by mouse sarcoma virus. The growth promoting activity was then eluted from the fixed monolayer by a pH change. A homogeneous preparation of SGF, biochemically and immunologically distinct from EGF, could be obtained after only two elution cycles. Interestingly, SGF and EGF differ in their biological activity. In contrast to EGF, SGF stimulates the normal fibroblasts to assume a 'transformed' morphology and to form colonies in semi-solid medium, a characteristic attributed only to tumour cells (G.J. Todaro, NIH, Bethesda). This elegant modification of affinity chromatography with fixed cells should certainly allow the discovery of other proteins able to trigger the initiation of DNA synthesis.

It is possible at this stage to distinguish two classes of cell growth promoting proteins, that is, those released by normal cells which could be considered as the normal growth regulators and those released by tumour cells or by virus-transformed cells. More detailed biochemical and immunological analyses are now required to bring some order into the classification of such macromolecular growth factors.

In addition, greater knowledge of their mode of action would allow a better classification. However, the following facts reported at this round table discussion illustrate the complexity of such studies.

For example, growth-stimulating molecules such as prostaglandin $F_{2\alpha}$ and FGF can act synergistically in 3T3 cells (V. Richmond, Friedrich Miescher-Institute, Basel).

Cells also release high and low molecular weight factors which stop the cell cycle and, therefore might be able to antagonise or alter the biological effects of the growth factors. For example, a macromolecule has been isolated which reportedly arrests cells in G_1 , an effect also produced by ACTH, thyrotropin or interferon (Holley).

Culture conditions also indirectly influence the sensitivity of cells to growth factors. For example, the number of receptors for EGF drops with increasing cell density and the binding kinetics are also affected (Holley). Moreover, viral transformation of cells can lead to a loss of EGF receptors (Todaro). It is also important to remember that cell growth regulation *in vitro* can be influenced by physical parameters which are not exactly similar to those predominant *in vivo*. For instance, the type of collagen used to coat Petri dishes can modify cell sensitivity to EGF and cell survival (W.R. Kidwell, NIH, Bethesda).

However, the macromolecular factors are apparently not solely responsible for the regulation of the initiation of DNA synthesis. Low molecular weight substances such as the classical hormones and nutrients (prostaglandin $F_{2\alpha}$, glucose,

inorganic salts and so on) are also able to potentiate the effects of macromolecules or to elicit growth alone (Richmond; Holley). The field is now entering a phase in which one might consider as a working hypothesis, the existence of metabolic steps which are simultaneously triggered by all these different substances to initiate cell division. Nevertheless, it remains possible that the regulation of cell growth is due to a delicate balance between many chains of events which are not all metabolically linked. □

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Tokamaks still to the fore

from R.J. Bickerton

AT the 9th European Physical Society Conference on Controlled Fusion and Plasma Physics* the tokamak continued to hold the centre of the stage. Tokamaks are toroidal discharges maintained to a satisfactory level of stability by the superposition of relatively strong toroidal fields. The system has many advantages — not least the simplicity and ease with which hot, well-confined plasmas can be obtained. It has however two serious disadvantages. Powerful additional heating is needed to get high temperatures and the ratio of plasma pressure to magnetic field pressure is usually very low. The heating requirement has been met in the past with outstanding success by injecting powerful beams of fast (~ 40 keV) neutral atoms. Record ion temperatures of the order 6 keV were achieved in this way last year with more than 2 MW of injected beam into the Princeton tokamak PLT. But the longer term prospects for this technique are dim because for reactor scale plasmas higher energy beams are needed, making it technically difficult, expensive and inefficient. Thus great attention was given at the meeting to the new results on radio-frequency heating. In one set of experiments a few hundred kilowatts of power at the frequency of ion cyclotron resonance were injected into the tokamaks TFR (Paris) and PLT. In both cases the ion temperature was increased by several hundred electron volts with an efficiency comparable to that of low energy neutral injection. In this frequency range (20-60 MHz) there is no technical problem in generating the power; the main difficulty is in coupling the power to the plasma. Heating of tokamak plasmas at other plasma resonance frequencies was also reported, the most notable being the use of

*A round table discussion organised by S. Iacobelli and L. Jimenez de Asua on 'Regulation of initiation of DNA synthesis and differentiation in cultured animal cells' was held in Rome on 8 October, 1979.

*Held in Oxford on 17-21 September, 1979.

100-200 kW of power at the first harmonic of the electron cyclotron resonance (~ 85 GHz) to raise the electron temperature in the Soviet tokamak T-10. In this frequency range the generation of high powers is the major difficulty; coupling to the plasma is through relatively simple wave-guide launchers. A general feeling at Oxford was that plasma heating in tokamaks by the injection of electromagnetic waves is at last becoming competitive with neutral injection. With the rich possibilities arising from a variety of frequencies it is hoped that an efficient method can eventually be found to deposit megawatts of power into the plasma core.

A second potential disadvantage of the tokamak arises from the same strong toroidal field that gives it its stability and general robustness. The ratio of plasma pressure to magnetic field pressure (β) tends to be very low, typically less than 1%. To be near economic a reactor needs β averaged over the volume ($\bar{\beta}$) to be in excess of 5%. New results included β values of the order 2-3%, seen in the ISXB (Oak Ridge), T-11 (Moscow) and TOSCA (Culham) devices. These values are obtained in circular cross-section devices and there are theoretical grounds for hoping that the higher values needed will be obtainable in non-circular cross-section systems such as JET. It should be emphasised that all three of these high β experiments are in effect limited by the available input power. Thus we only know that the values of β at which instability leads to a decrease in confinement are greater than those seen so far. Apart from these advances in tokamak heating and $\bar{\beta}$ there were many other results reported — notably the operation of the large Soviet tokamak T-10 at low values of q (~ 1.6) without a decrease in the confinement properties.

First results were reported from the new ETA-BETA II reversed field pinch experiment at Padua. This is approximately a one-third scale version of ZETA with some additional control of the toroidal magnetic field. To the surprise of some, the main features of the so-called quiescent period in ZETA have now been reproduced in this smaller device. Fluctuations in dI/dt are markedly reduced following field reversal and the electron temperature rises during quiescence from ~ 40 eV to ~ 100 eV. It will now be possible to study this quiescence phase in detail with modern diagnostics and hence to assess the potential of the reversed field system.

Research on the mirror system of magnetic confinement is now concentrated on two versions. The first is one in which the initial field of the mirror is reversed by plasma currents to give a final configuration which is similar to a compact tokamak without toroidal field and which has closed field lines and is therefore not a mirror at all. This is being studied at the Lawrence Livermore Laboratory and so far all attempts to achieve field reversal by pumping up the plasma pressure have

marginally failed. New experiments are planned in which a low temperature plasma with field reversal is first created by a plasma gun and then heated by intense neutral beams. The second version is the so-called tandem mirror in which a long straight solenoid has intensely heated minimum B mirrors at each end. This line is also being studied at Livermore as well as in Japan and Novosibirsk. The aim of the tandem mirror is to create an electrostatic potential at the ends of the solenoid in such a way as to confine the ions. A major difficulty with all these open-ended systems is the heat conduction along the field lines by the highly mobile electrons. First results from the Livermore tandem mirror TMX were presented at Oxford. These show encouraging evidence of electrostatic plugging and of effective electron heat confinement. Thus the electron temperature in an end mirror is ~ 160 eV while in the solenoid the ion temperature is low (< 100 eV), the density $\sim 10^{13}$ cm $^{-3}$ and the end loss small.

In the field of inertial confinement attention is now focused on ablative compression of complex pellets using as driver systems either lasers, electron, light ion or heavy ion beams. In the USA 10 kJ, 20 TW lasers at 1 and 10 μ m wavelengths are now operating at Livermore and Los Alamos respectively. With the big Livermore laser (SHIVA) the D-T fuel has been compressed to ~ 160 times liquid density. These ablatively compressed cores are relatively cold and the next step will be to increase the implosion velocities through

the use of higher power lasers on to larger targets. An interesting technique reported at the meeting was the use of radiochemical methods to deduce the density of the compressing shell. Transmutations are produced by the neutrons from the core and the subsequent radioactive decay is measured.

The choice of the best system is still open with lasers clearly ahead for research purposes but with embarrassingly low efficiencies for an ultimate reactor. All the particle drivers are more efficient but there are greater difficulties in transporting and focusing them to a target. If the energy gain from a single implosion can be made large enough then the efficiency of a CO $_2$ laser (1-5%) may be tolerable. Such high gains (~ 100) are theoretically possible from complex targets, but there is then the problem of making them cheaply.

For the first time in this series of conferences there was a report on the fusion programme in the People's Republic of China. Many were surprised by the size, breadth and sophistication of this work which includes mirror machines, laser compression and several tokamaks. The programme is gathering momentum after the setbacks of the Cultural Revolution. The presentation at Oxford was limited to a catalogue of devices but we can expect a serious contribution to fusion physics from China in the near future. $\square$

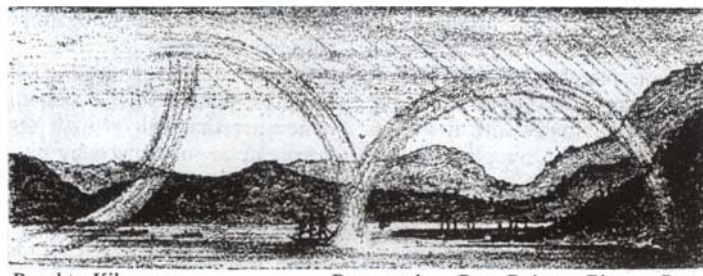
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Erratum

During printing a line was inadvertently omitted from the article 'Road to unification' (*News & Views* 282, 131; 1979). The first sentence in column 3, page 132 should read "Experimentally, weak neutral currents were discovered at CERN in 1973¹⁹ and parity-violation in neutral currents of precisely the right size was discovered in the remarkable experiment²⁰ at SLAC last year." Our apologies to CERN.



100 years ago



Road to Kilcreggan.

A

B

C

Roseneath. Row Point. Pier. Row.

D

A curious rainbow

I send you a rough sketch of a curious rainbow group seen in Gareloch about 8.25 a.m. on October 20. I would have written sooner but I delayed till I had obtained sketches from several different sources. I only saw the junction of the two bows at C, that

being the only part of Row Bay visible from my standpoint, but several observers saw the whole group as I have drawn it. The sea was quite glassy, so that the inverted rainbow A must have been formed by the sun's rays reflected from the water. The wind was just beginning to rise and some scudding showers were passing up from the Firth of Clyde from the south-west, but the bay was quite calm.

The bow D was perfectly full and bright, while B died away at its highest point. I can only imagine that B was formed by light reflected by some bright cloud, but I did not observe any bright enough. The view is nearly north-west.

From *Nature* 21, 20 Nov., 56; 1879.

review article

Brownian motions of flexible polymer chains

P. G. de Gennes

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The dynamics of flexible chains in solutions and in melts has recently been investigated using photon beat methods, inelastic scattering of neutrons, and also the stimulated Rayleigh effect. Relatively simple scaling laws have emerged, but some features of entangled systems remain unexplained.

THE stochastic motions of flexible polymer chains in a liquid phase are very complex. They fall into two classes: first, local motions, involving conformational changes inside a small number of adjacent monomers; second, large scale motions, where the whole polymer chain deforms and drifts in the neighbouring solvent.

The frequencies involved are in the range 10^{10} cycles for local motions, and $1-10^5$ cycles for large scale motions. Here only the latter will be considered. These relatively slow modes have mostly been studied through mechanical experiments^{1,2}. A very small concentration of flexible polymer is sufficient to increase significantly the static viscosity of a solvent; if we repeat this measurement at higher frequencies (using acoustic shear waves) we find an elastic component to the response with the chain behaving like an elastic spring. The frequency dependence of the response indicates a certain relaxation time of the chains. A similar time can be measured using certain mechano-optic and dielectric effects³.

The 'photon beat' method has allowed the study of large scale motions. Here a very monochromatic laser beam (of frequency ω_0) is scattered inelastically from the polymer coils and the outgoing beam (frequency $\omega_0 + \omega$) is probed at a well defined angle θ (or scattering wave vector $q = 4\pi \sin \theta / \lambda$). The useful range of $\omega/2\pi$ is $1-10^6$. The new feature of this method is the choice of a wave vector q . Qualitatively we may say that this method probes local fluctuations in the polymer concentration at spatial scales of order q^{-1} (ranging from 1,000 Å to a few microns).

Experiments using the weakly inelastic scattering of neutrons^{4,5} have also become feasible; they give similar information, but for higher frequencies: from 10^{11} s^{-1} with 'back scattering spectrometers' down to $2 \times 10^8 \text{ s}^{-1}$ with the most recent 'spin echo' method. The range of q vectors available with neutrons is also different: for most experiments q^{-1} is between 10 and 100 Å. From these optical and neutron data, a physical picture has emerged for the stochastic motions of polymer chains, which is summarised below.

Dynamics of a single chain in solution

When considered at small magnification (low q) a polymer coil behaves like a point-like brownian particle, with a certain diffusion coefficient D : this is conveniently measured by photon-beat experiments. For flexible coils in good solvents, D is found to be a decreasing function of the molecular weight M , and is often well described⁶ by a relationship of the form

$D \sim M^{-x}$ where x is of the order of 0.55. This does not agree with the classical theory for D (due to Kirkwood⁷) which predicts a Stokes-Einstein form:

$$D = \frac{kT}{6\pi\eta_0 R} \quad (1)$$

where T is the temperature, η_0 is the solvent viscosity, and R an effective radius of the coil. In a good solvent we would expect R to be controlled by excluded volume effects (giving a swollen coil): this gives $R \sim M^{3/5}$. The discrepancy in the exponent is beyond experimental errors, and remained a mystery for a long time until an attractive explanation was proposed by des Cloiseaux and Weill⁸. They note that in most of the usual solvents, which are not 'very good' the pair correlation function $g(r)$ between monomers of the chain, is of the ideal chain form [$g(r) \sim 1/r$] for short distances $r < r_c$, while $g(r)$ decreases more rapidly at larger r : $g(r) \sim \text{const } r^{-4/3}$ ($R \gg r \gg r_c$). This 4/3 law is the classic result due to Edwards⁹, and reflects excluded volume effects typical of good solvents. The length r_c has been displayed in certain neutron diffraction experiments^{10,11}, and may be of the order of 20–100 Å (depending on the solvent quality). The main observation is that all static determinations of the coil radius are derived from an average of r_{ij}^2 between two arbitrary monomers

$$R_{\text{stat}}^2 \sim \int r^2 g(r) \text{d}r / \int g(r) \text{d}r \quad (2a)$$

while the dynamic properties are dominated by backflow properties⁷: the motion of monomer (i) induces a flow field near monomer (j) proportional to $1/r_{ij}$. This means that

$$\frac{1}{R_{\text{dyn}}} \sim \int \frac{1}{r} g(r) \text{d}r / \int g(r) \text{d}r \quad (2b)$$

The static average, equation (2a), is dominated by the contributions at large r , for which the excluded volume law behaves well. But the dynamic average, equation (2b), is more sensitive to the short range portion of $g(r)$, and for chains of not too large M , this makes R_{dyn} closer to the ideal chain value ($\sim M^{1/2}$). (Ultimately, at very high M , when $R \gg r_c$ the two values converge $R_{\text{dyn}} \sim R_{\text{stat}} \sim M^{3/5}$.) Future experiments using solvents of variable quality (variable r_c) should test the des Cloiseaux-Weill explanation in detail.

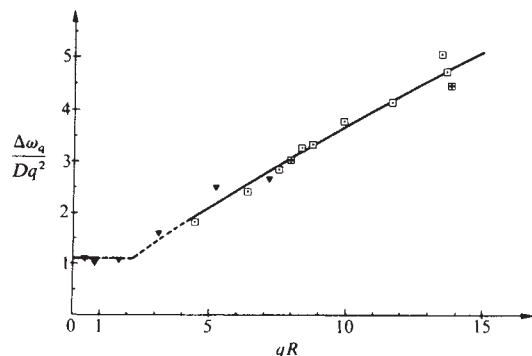


Fig. 1 Characteristic frequency $\Delta\omega_q$ for fluctuations of wavelength $2\pi/q$ in dilute polystyrene solutions. A scaling law is constructed by plotting $\Delta\omega_q/Dq^2$ versus qR where R is the coil radius and D the diffusion coefficient. Note that the points measured for two molecular weights (∇ , $M = 8 \times 10^6$; $\square$, $M = 24 \times 10^6$) fall on one curve (after ref. 12).

The motions of the chain can be described in terms of an overall diffusion only when the probing length (q^{-1}) is much larger than the coil size R . The opposite limit has been studied¹² in very large polystyrene coils (with M values up to 2×10^7). The resulting plots of inelastic linewidth $\Delta\omega_q$ versus q (Fig. 1) can be summarised in the form:

$$\Delta\omega_q = Dq^2 f(qR) \quad (3)$$

where f is a dimensionless function. The long wavelength limit ($qR < 1$) is the diffusion law $\Delta\omega_q = Dq^2$ and imposes $f(0) = 1$. At the other end ($qR \gg 1$) we expect $\Delta\omega_q$ to become a characteristic frequency for a chain portion of spatial extension q , and to be independent of the overall chain size R . Because $D \sim 1/R$ from equation (1) then $f(x)$ must be linear in x for $x \gg 1$, giving:

$$\Delta\omega_q = \text{const} \frac{kTq^3}{\eta_0} \quad (qR \gg 1) \quad (4)$$

This scaling law is followed relatively well by the data (although in practice the comparison is complicated by the des Cloiseaux-Weill correction). Theoretically, equation (4) was originally derived using a special model¹³ for ideal chains, but it has a more general range of validity. Note the analogy between equation (4) and the rotational relaxation rate for a sphere of radius q^{-1} [$1/\tau_q = (kT/8\pi\eta_0)q^3$].

Some measurements of $\Delta\omega_q$ have also been obtained from inelastic scattering of neutrons. As already mentioned, the results are limited to rather large $\Delta\omega_q$ values, and this imposes relatively high q : the data always fall in the domain $qR \gg 1$. Early work^{14,15} was mainly concerned with melts and concentrated solutions, for which the situation is complex. Recent studies, using the 'spin echo' frequency analyser, have been performed on solutions of polydimethyl siloxane and polymethyl methacrylate, in benzene¹⁶. They give $\Delta\omega_q \sim q^z$ with z close to 3—in reasonable agreement with equation (4). Typical values of q are of the order of $0.1 \text{ \AA}^{-1}$, hopefully small enough to warrant the continuum description (Fig. 2).

The above discussion on $\Delta\omega_q$ has been restricted to good solvents, where the chains were strongly swollen. In early theoretical discussions² the emphasis was more on the so-called 'Θ solvents' where the steric repulsions between monomers are exactly compensated by Van der Waals interactions: this led to simple random flight statistics for the coils ($R \sim M^{1/2}$) and eliminated the complications of excluded volume effects. However, another difficulty appears (at least for very long chains) in Θ conditions: as each coil is less swollen, it has a strong tendency to become knotted on itself. These knots could complicate the dynamics, the coil behaving as a whole like a small rubber ball¹⁷. Present experiments, however, have not shown any clear-cut effect of knots in Θ solvents, and this point remains unclear.

Diffusion in semi-dilute solutions

Let us now consider many coils in a good solvent, with a concentration c (g cm^{-3}) such that different coils overlap very strongly. The internal concentration of a single coil is of the order of $c^* = MR^{-3} \sim M^{-4/5}$ and the present regime corresponds to $c \gg c^*$. For large M values c^* is very small, and, the condition $c \gg c^*$ is achieved for a broad range of 'semi-dilute' solutions.

In these solutions, we can define two distinct diffusion processes: (1) 'cooperative diffusion' (D_{coop}) is associated with long wavelength fluctuations of the polymer concentration, and can be probed by photon beat experiments¹². For instance, with polystyrene, in relatively good solvents, one finds a value of D_{coop} which is independent of molecular weight and increases with concentration: $D_{\text{coop}} \sim c^m$ with $m \sim 0.67$.

These are natural features: all the chains drift simultaneously, and behave as an elastic 'sponge' moving in a viscous medium. The size of the holes in the sponge (called the correlation length ξ (ref. 18) depends on c but not on M : both the elastic restoring forces and the friction forces involve only ξ and are independent of M in the semi-dilute regime: the same holds for D_{coop} . Furthermore, when c increases, the elastic rigidity of the 'sponge' increases very fast, and this explains the increase in D_{coop} .

It is easy to construct scaling laws for ξ and for D_{coop} (ref. 19). For the correlation length:

$$\xi = R \left(\frac{c}{c^*} \right)^n \quad (c \gg c^*) \quad (5)$$

noting that for $c = c^*$ (close packed system of non-overlapping coils) the size of the interstices is comparable to the single coil radius R . Furthermore, we want ξ to be independent of M . Since $R \sim M^{3/5}$ and $c \sim M^{-4/5}$ this means that $n = 3/4$, a value confirmed by neutron diffraction data¹⁸. Similarly for the cooperative diffusion coefficient we can write:

$$D_{\text{coop}} = \frac{T}{6\pi\eta_0 R} \left(\frac{c}{c^*} \right)^{3/4} = \frac{T}{6\pi\eta_0 \xi} \quad (6)$$

Equation (6) ensures that, for $c \sim c^*$, D_{coop} returns to the single coil value of equation (1) and the exponent is such that D_{coop} is independent of M . The remaining discrepancy between the theoretical exponent (3/4) and the experimental value for polystyrene ($m = 0.67$) is probably another effect of the des Cloiseaux-Weill correction.

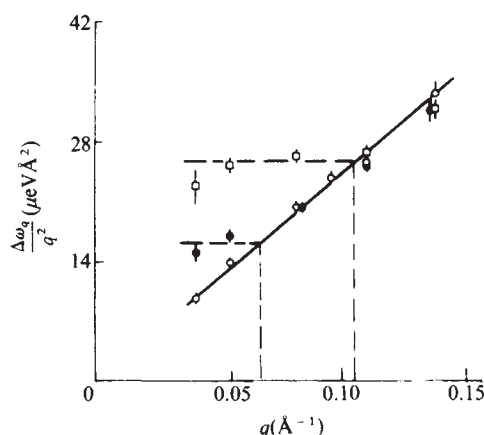


Fig. 2 Plot of $\Delta\omega_q/q^2$ versus q for three solutions of polydimethyl siloxane in benzene $\circ$, PDMS/B 0.05 g; $\bullet$, PDMS/B 0.15 g; $\square$, PDMS/B 0.3 g (after ref. 16). Note the difference in q values with Fig. 1: neutrons probe higher q s. The relevant curve of the present discussion corresponds to the most dilute solution ($\circ$). At higher concentrations and low q values ($q\xi < 1$) a more complicated regime appears.

The friction factor between the 'sponge' and the solvent can also be measured directly by sedimentation studies; data on polymer solutions have been collected for a long time²⁰ but it is only recently that a systematic study in semi-dilute solutions of low c has been carried out²¹. The sedimentation coefficient of polystyrene in good solvents is found to decrease like $c^{-0.59}$ while scaling would predict $c^{-0.5}$.

(2) 'Individual diffusion' (D^*) is associated with the brownian motion of one labelled chain immersed in a solution of chemically identical (but unlabelled) other chains. The diffusion coefficient D^* is rather small and very different from D_{coop} : it is a decreasing function of concentration and also of molecular weight. D^* is controlled by certain wiggling motions of the probing chain among the other chains, all being strongly entangled; therefore a theoretical study of D^* is difficult. I suggested previously²² a simpler physical situation, where a single mobile chain moves in a fixed network of other chains: this has been called reptation. For the reptation model, D^* is proportional to M^{-2} . Various realisations of the model have been achieved²³. However, the simplest idea was realised only recently: it replaces the network by a solution of 'ambient' chains, chemically identical to the probing chain, but longer: then the motions of the ambient chains are so slow that effectively they behave like a fixed network.

Two main experimental approaches have been used to study the very slow diffusion described by D^* . In the Klein experiments²³ the labelled chains were deuterated, and their concentration was measured by IR spectroscopy. The diffusion times involved were measured in months, and the diffusion lengths were ~ 2 mm. In recent experiments by Hervet, Léger and Rondelez²⁴, a photochrome was attached to the probing chain, and the diffusion was monitored through the stimulated Rayleigh effect.

The principle is as follows: at time 0, with a strong laser pulse, we create an interference fringe pattern (of wavelength $2\pi/q$) in the solution. The photochrome molecules are chemically modified in the bright regions, and this creates a periodic modulation of the refractive index; a phase grating is constructed in the fluid. We then cut out the power laser; the amplitude of the refractive index modulation Δn at a later time t decreases like $\exp(-D^*q^2t)$ where D^* is the diffusion coefficient. We monitor this amplitude with a probing (weak) laser, measuring the intensity of a Bragg reflection on the grating. This method detects diffusion over very small lengths ($2\pi/q \sim 1 \mu\text{m}$) and thus allows measurements of very small diffusion coefficients D^* (down to $10^{-9} \text{ cm}^2 \text{ s}^{-1}$ at present). The main difficulty in this programme is chemical: the attachment of a photochrome at the end of a polystyrene chain, prepared by anionic polymerisation is a delicate operation (performed under direction of P. Rempp). The labelled chains and the ambient chains were both polystyrenes of the same length. (This is not the ideal reptation situation, where the ambient chains would be longer than the probe. However, it has been argued that the difference is not essential^{25,26}.) It was found that $D \sim c^{-\alpha}$ where $\alpha = 1.7 \pm 0.1$.

It is possible to predict an exponent of this magnitude by combining the rules of reptation ($D^* \sim M^{-2}$) with a scaling ansatz:

$$D^* = \frac{T}{6\pi\eta_0 R} \left(\frac{c}{c^*}\right)^{7/4} \quad (7)$$

The form of equation (7) ensures that D^* crosses over correctly to the single coil D at $c = c^*$. The exponent 7/4 is then chosen to make $D^* \sim M^{-2}$.

In spite of its apparent success, equation (7) must still be considered with some suspicion: (1) the self diffusion D^* is associated with a natural relaxation time τ , proportional to $R^2(c)/D^*$ (where $R(c)$ is the size of the chain at concentration c). Using known scaling laws for $(R(c))$ (ref. 2) this leads to $\tau \sim M^3$ (a typical reptation result^{27,28}) (Fig. 3). However, most mechanical data on τ , (ref. 1) suggest a higher exponent $\tau \sim M^{3.3}$. The discrepancy remains completely unexplained at

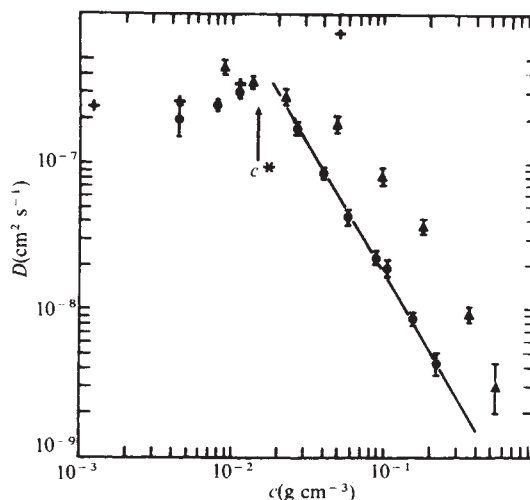


Fig. 3 Self diffusion coefficient D^* versus concentration in polystyrene-benzene solutions for two molecular weights (after ref. 24). Δ , $M = 123,000$; $\bullet$, $M = 245,000$; +, calculated from ref. 7; slope $\alpha = -1.75$.

present. (2) Similar scaling arguments could be applied to the viscosity η of the entangled solution: making η equal to the solvent viscosity η_0 when $c \leq c^*$, and demanding that η be simply proportional to τ , (to M^3) in melts. One would then reach

$$\eta \cong \eta_0 \left(\frac{c}{c^*}\right)^{15/4} \quad (8)$$

The actual exponent of $\eta(c)$ has not been measured in many clear-cut cases with low c^* values; but recent data (M. Adam and M. Delsanti, personal communication) suggest a higher exponent (~ 4.5). Thus either the scaling ansatz, equation (8), is wrong, or some minor complications occur: one of them may be due to a competition between monomer-solvent friction (described by η_0) and monomer-monomer friction: in equation (8) we might have to make the replacement

$$\eta_0 \rightarrow \eta_0 + \eta_1 c + \dots \quad (9)$$

where η_1 describes monomer-monomer friction (and is independent of M); if the term $\eta_1 c$ dominates on η_0 , even at relatively low c , the apparent exponent in equation (8) may be modified. However, if we accept this idea, we probably need a similar renormalisation of η_0 in equation (7) which might upset the agreement with the experiment.

Conclusion

The combination of the classical (mechanical) data on polymer dynamics, and other information (optics, neutrons) produces a reasonably consistent description of the long range, low frequency motions of polymer coils. However, (1) certain features, like the des Cloiseaux-Weill correction, or the viscosity renormalisation of equation (9), complicate the situation; (2) there remain some mysteries such as the possible role of knots in Θ conditions, or the exponent for the relaxation time $\tau_r(M)$ in entangled systems. The problem raised by $\tau_r(M)$ is central: as mentioned earlier, the experimental relaxation times are longer than expected. Polymers of high molecular weight may never be absolutely linear, but rather show some branching. Branched species cannot reptate freely, and are expected to have exponentially long relaxation times. One long branch is enough to achieve this; and a single branch point should be invisible by all traditional methods of detection (UV, IR spectroscopy, and so on).

Thus mechanical data on long, entangled chains may be dominated by a small number of uncontrolled branch points. However, this explanation is difficult to reconcile with the

apparent universality of the result: $\tau_r(M)$ is proportional to $M^{3.3}$ or $M^{3.4}$ for a wide variety of chemical structures.

Apart from branching effects, polydispersity may also be important: if the sample has a distribution of molecular weights M , the mechanical data in the entangled state are extremely sensitive to the high M tail of the distribution. We hope to see

this point clarified by systematic studies on blends with a small fraction of very long chains in a matrix of smaller chains²⁶.

This review is largely based on a workshop on polymer dynamics held in Les Houches 1979 under the direction of J. P. Cohen Addad, and I thank the participants for many stimulating discussions.

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articles

Thermal minima along the axis of the Mid-Atlantic Ridge

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Mid-Atlantic Ridge basalts erupted near fracture zones seem to have lower quenching temperatures than along unfractured ridge segments probably because of enhanced cooling by exposing magmas to colder fault planes and more intense seawater circulation. The cooling effect is most pronounced along segments over hotspots with close ridge displacement fabric and recurrent rift jumping, probably reflecting additional complications, including subaxial magma flow about 'plumes', or the presence of an unusually large magma chamber on the Azores Platform and the southern part of the Iceland Plateau.

LARGE-SCALE heterogeneities in the abundance of heat-producing elements in the mantle should generate varying heat production rates. Given sufficient time after such heterogeneities have been produced, unusual melting conditions or convective flow patterns may develop in regions of the mantle richer in U, Th and K. Surface reflection of such unusual mantle activity may be the locus of the so-called 'hotspots' such as Iceland, the Azores and Galapagos regions which are characterised by more vigorous volcanic activity than adjacent mid-ocean ridges. The progressive increase in the content of K, U, Th of basalts collected along the Reykjanes Ridge and Iceland may reflect such mantle heterogeneity^{1,2}. On average, Iceland basalts are enriched in K, U and Th by factors of 3.8, 6 and 9.6, respectively, compared with Mid-Atlantic Ridge basalts located south of 61° N. A significantly higher heat production rate is thus expected beneath Iceland relative to the Mid-Atlantic Ridge further south. However, it is uncertain whether such a higher heat production rate would manifest itself in generating higher

heat flow, greater extent of melting, higher magma temperatures, unusual mantle convective flow, or a combination of these phenomena. It is also not known when these mantle heterogeneities were produced. High heat flow has been measured over Iceland³ and steeper geothermal gradients have been inferred for Iceland compared with the Mid-Atlantic Ridge⁴⁻⁶, but no direct heat flow measurements are yet available on the Mid-Atlantic Ridge crest. Lava production rates over Iceland are two to four times higher than on the Mid-Atlantic Ridge judging from crustal thickness and the Iceland topographic bulge^{3,7-9}. This would indicate higher convective heat transport beneath Iceland than along the Mid-Atlantic Ridge. However, it is uncertain whether the higher basalt production rate on Iceland represents a higher degree of melting (melt-solid mass ratio) or segregation of interstitial melts from a larger volume of mantle rock beneath Iceland.

Lava temperatures are another useful way of investigating thermal phenomena, although also limited. Lava temperatures, whether directly measured or calculated from petrological data, only reflect temperatures of the magma at the time of eruption. The temperature of primary magma generated in the mantle is affected by cooling, partial crystallisation and possibly volatile loss as the magma ascends. The extent of polybaric fractional crystallisation of primary melts during ascent is a source of debate. While some workers maintain that all basalts have suffered extensive fractionation¹⁰ others consider that some basalt compositions reflect relatively primary liquid¹¹. Despite these limitations the determination of liquidus temperatures of basalts erupted along the Mid-Atlantic Ridge encompassing hotspots or fracture zones can be useful^{12,13}. An estimation of the variation of temperature of magmas along the ridge may not only provide clues on thermal conditions in the underlying mantle, but also clarify the relative ascent rate and cooling

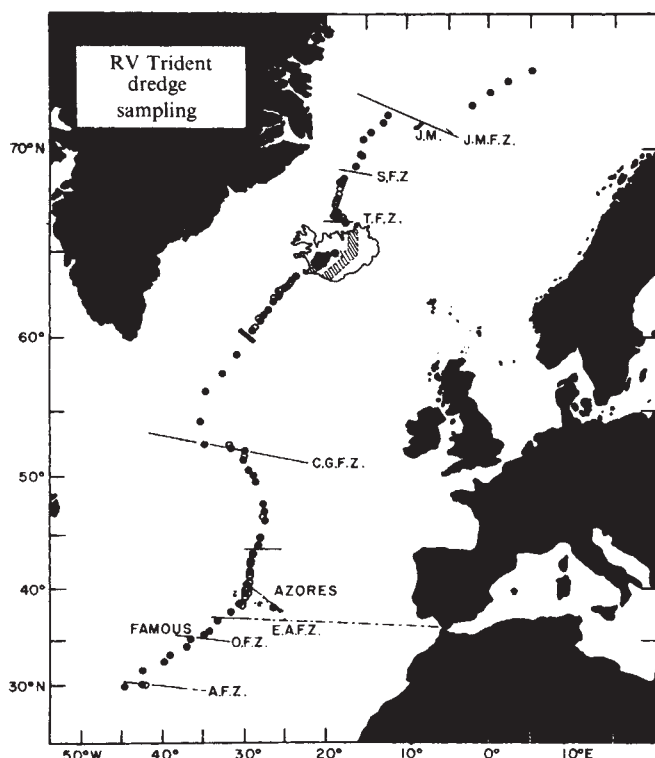


Fig. 1 Location of the Mid-Atlantic Ridge dredge stations used for the study (●). Not all locations contained glasses.

history of magmas in relation to tectonics. Further comparison of liquidus temperature variations along the ridge with other petrological and geochemical data, including heat producing elements U, Th and K, should also help to develop more realistic models for magma emplacement, ridge evolution and formation of unusual oceanic plateaus such as the Azores, Iceland and Jan Mayen. The first use of such an approach was made by Hermes and Schilling¹² along the Reykjanex Ridge and Iceland.

Here we examine the variation in calculated olivine crystallisation temperatures of 100 young Mid-Atlantic Ridge basalt glasses along the axis of the ridge from 29° N to 73° N, including traverses over Iceland (13 sub-glacial pillow lava localities from the western volcanic zone) and the Azores Plateau (Fig. 1). The submarine glassy rocks were dredged by RV Trident from 66 stations located either within the rift of the Mid-Atlantic Ridge or on the axis of the elevated crest of the Reykjanex and the Kolbeinsey Ridges. The stations are spaced at an average interval of 55 ± 63 km (1σ) and provide a reasonably representative sampling of recent magmatic activity on a 4,900-km long segment of the mid-ocean ridge system. Whenever possible, several samples were studied per station¹⁴. Liquidus temperatures were computed on the basis of the temperature-dependence of the equilibrium partitioning of Mg between olivine and co-existing glass¹⁵⁻¹⁸. The olivine crystallisation temperatures reported here are all based on the rim composition of olivine 'microphenocrysts' and surrounding glass in quenched pillow lava rinds and are considered to be realistic approximation of temperatures of Mid-Atlantic Ridge basalts at or close to the time of extrusion. Detailed discussion of this electron microprobe study, including the methods and the validity of the calculated temperatures of olivine crystallisation, are reported elsewhere¹⁴.

Petrology

Phenocrysts are present in the majority of the glassy basalts studied. Modal analyses show that total phenocrysts are generally <10 vol.% in the variolitic zone of the pillows, with maximum phenocryst content up to 23% near Iceland, the Azores Plateau and the Gibbs Fracture Zone (Fig. 3). Plagio-

cline phenocrysts are generally more abundant than olivine, and clinopyroxene is uncommon except over and near the Azores, Iceland and Jan Mayen plateaus. The composition of the olivine microphenocrysts studied ranges from 71.8 to 88 mol% forsterite, with a mode at 85 mol% Fo. A good linear relationship exists between the Fe/Mg content of these olivines and that of the glass. The corresponding K_D equal to 0.27 ± 0.01 , 1σ suggests that equilibrium conditions prevailed¹⁴. (The $\text{Fe}^{2+}/\text{Fe}^{3+}$ values of the glasses were estimated on the basis of an oxygen fugacity equivalent to that of the quartz + magnetite-fayalite exchange reaction at the liquidus temperature calculated from the Mg partitioning, using Waff and Weill's empirical relationship (unpublished data). The $\text{Fe}^{2+}/\text{Fe}^{3+}$ obtained this way compared well with those actually measured on whole rock by a wet chemical method (Schilling *et al.* unpublished).

Within the normative classification scheme of Yoder and Tilley¹⁹ the bulk compositions of the basalts studied correspond to either quartz-hypersthene or olivine-hypersthene normative basalts, and only two Ne-normative lavas were observed near 33–35° N. Stations TR119-8D recovered basalts which are just nepheline normative, and the glassy rims of picritic basalts at station TR123-4D are also just nepheline normative whereas the whole-rock is not. As expected, glass compositions are slightly more evolved than bulk rocks. Silica content of glasses ranges from 47.4 to 53.1% (mean 50.9 ± 1.0 , 1σ) and in bulk rock analyses from 46.9 to 52.0% (mean 49.9 ± 1.0 , 1σ). The chemistry of these pillow lavas, as well as of the glasses, reveals two major compositional groups which may be related to distinct petrological provinces (Fig. 2). One group forms a narrow iron enrichment trend with little change in alkali content for the region of the Mid-Atlantic north of the Gibbs Fracture Zone up to 70° N including Iceland (group A); the other group shows a notable enrichment and significant scatter in soda and comparatively more limited Fe enrichment and comprises basalts from the segments of the Mid-Atlantic Ridge between 53° N to 28° N including the Gibbs Fracture Zone and the Azores Plateau, as well as from 70° N to at least 73° N, which includes the Jan Mayen region and the Mohns Ridge (group B). Group B basalts can further be subdivided into ridge segments with distinct content of soda at equivalent Mg-value. The difference between the two groups is amplified in the more evolved magmas. The forsterite content of olivine from group A varies from 87 to 72 mol% Fo, and from 88 to 74 mol% Fo for group B. At equivalent olivine compositions, the Mg and Fe content of group

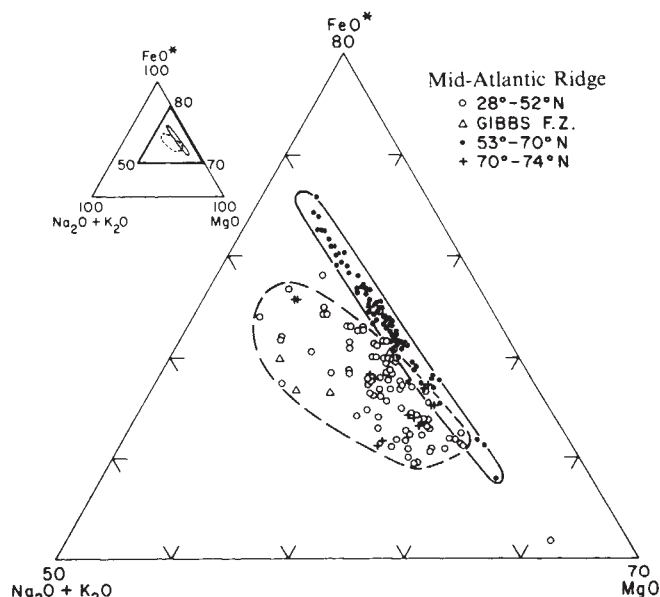


Fig. 2 Relative Mg, Fe, alkali content variation of the Mid-Atlantic Ridge basalt studies based on whole-rock analyses. Not all basalts are glassy.

A glasses is consistently higher than in glasses of group B, over the entire compositional range. Consequently, the olivine/glass partition coefficients for both Fe and Mg are higher in group A than in group B, but the corresponding K_D values for the two groups are indistinguishable within the error quoted earlier. The differences in olivine/glass partitioning of Fe and Mg between groups A and B can be attributed to melts composition, particularly differences in SiO_2 (or Si/O ratio) which is generally higher in group A basalts at equivalent indices of fractionation¹⁴. These observations also suggest that the Fe and Mg partition coefficients are affected to a similar extent by composition, since K_D is the ratio of these two partition coefficients.

Temperature variations along the Mid-Atlantic Ridge

We have calculated the crystallisation temperatures of olivine microphenocrysts in Mid-Atlantic Ridge basalt glasses by using the temperature-dependency of the olivine/melt (glass) partition coefficient (D) for Mg defined as D_{Mg} = weight fraction of Mg in olivine/weight fraction Mg in liquid. The temperature dependency of the Mg partition coefficient has been experimentally determined at one atmosphere under controlled fugacity of oxygen for a wide range of basaltic compositions¹⁵⁻¹⁸. The relationship is of the form: $\log D = A/T \text{ (K)} + B$. These studies have also shown that the compositional dependency of D_{Mg} is small. The pressure dependency remains poorly studied. We have calculated the temperature of crystallisation of the olivine 'microphenocrysts' using the one-atmosphere D_{Mg} calibration of Fisk *et al.*¹⁸. This calibration was obtained on the Reykjanes Ridge basalts, which should further minimise possible compositional dependency of D_{Mg} in the case of the Reykjanes Ridge glasses and other glasses from group A. There are several uncertainties in the calculated temperatures of olivine crystallisation: (1) uncertainties in parameters A and B for the linear regression correlation between D_{Mg} and temperature can be as large as 30°C between various calibrations¹⁵⁻¹⁸; (2) uncertainties due to analytical precision for MgO may translate into $\pm 5^\circ\text{C}$ error on the calculated temperatures; (3) uncertainties due to the compositional variation of individual olivine microphenocrysts has been examined by compilation of average core and rim compositions for 144 Mid-Atlantic Ridge basalts as well as intensity line scans for Fe and MgK α across selected microphenocrysts. The number of olivine analyses per sample ranged from a minimum of 6 to >20. Zoning was taken to be significant when the difference in core and rim composition exceeded the precision of the microprobe analysis method ($\pm 0.25 \text{ mol}\% \text{ Fo}$). On this basis 40% of the samples have olivine microphenocrysts showing significant zoning. Of these, 49 samples exhibit normal zoning (decreasing forsterite content from core to rim) amounting to average of 1.4 mol% Fo, which corresponds to an $\pm 5^\circ\text{C}$ error on the calculated temperature. Reverse zoning was detected in 10 basalts, with average range of 0.75 mol% Fo. Maximum normal zoning observed was 2.82 mol% Fo (or an absolute uncertainty of 10°C on calculated temperature), and maximum reverse zoning of 1.50% (a 6°C absolute uncertainty). The composition of the glasses was determined by a minimum of three probe analyses of the glass, well away from crystal phases. Only average compositions of olivine 'microphenocryst rims' were used to calculate the Mg partition coefficients.

Variations in temperature of crystallisation of olivine along the ridge using this method are shown in Fig. 3. Based on our analysis of errors and uncertainties and systematic analytical procedure, we estimate that individual temperatures shown in Fig. 3 should not deviate by more than $\pm 8^\circ\text{C}$ on a relative basis. The temperatures range from a maximum of 1,225°C in an Icelandic picritic glass (IC-117) to a minimum of 1,067°C in quartz-normative tholeiite glasses on the southern margin of the Azores Plateau at 38°N (TR154-10D). These values are within the range of liquidus temperatures of basic igneous rocks determined in the laboratory²⁰. Temperature variations on a

single site were evaluated from data on glasses taken in 15 dredges from which two or three samples were selected for study. The temperature difference among several glasses from a single dredge varies from zero to 33°C and two groups of sites can be recognised. Five of the dredge stations contain heterogeneous samples, with dissimilar glass compositions, petrography and crystallisation temperatures; with temperature differences between samples of 11–33°C. These dredges have clearly sampled several unrelated volcanic units. Ten dredges, for which multiple temperature data are available, contain samples with temperature differences in the range 0–6°C. Other criteria, such as glass chemistry and petrography, indicate that these dredge stations contain several samples of the same volcanic unit.

Basalts with highest olivine crystallisation temperatures at each site have been joined by a line to yield a temperature profile of the ridge (Fig. 3). Also shown in Fig. 3 are: a bathymetric profile of the ridge based on water-depths at the dredge stations; the Mg-value of the basalts; the volume % of phenocrysts based on modal analyses (Schilling *et al.* in preparation), and the La/Sm variation as an index of mantle source heterogeneity^{1,21}.

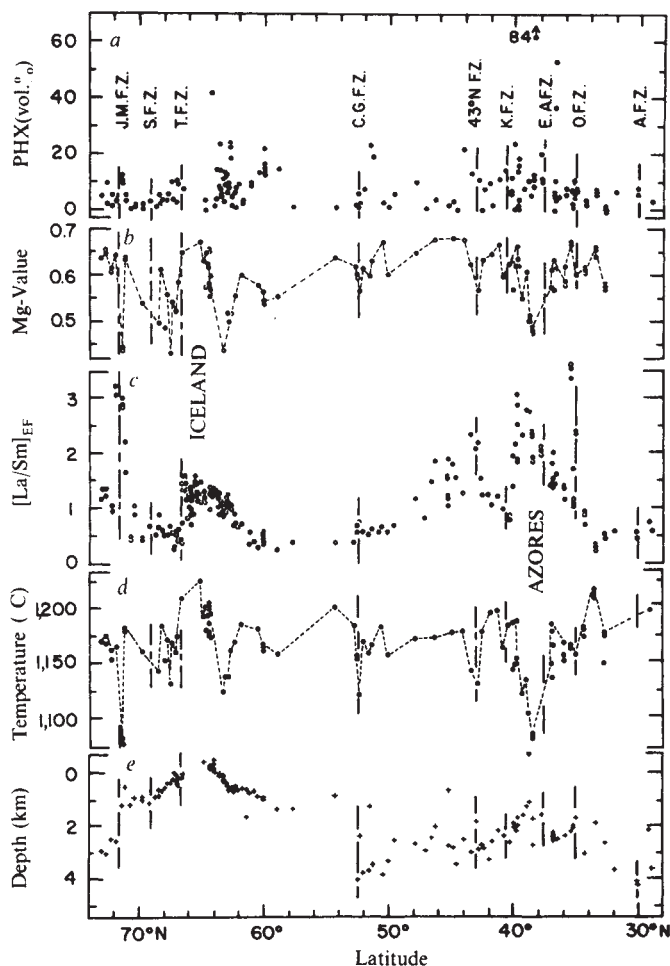


Fig. 3 Variation along the Mid-Atlantic Ridge, from top to bottom of: (a), total phenocryst volume per cent based on point counting modal analyses (Schilling *et al.* in preparation); b, Mg/Mg + Fe²⁺ of glasses (atomic and using the method described in the text for estimating the Fe²⁺/Fe³⁺ ratio)¹⁴; c, (La/Sm) enrichment factor relative to chondrites (refs 1 and 21 and Schilling *et al.* in preparation); d, calculated temperature of olivine and glass¹⁴, using the calibration of Fisk *et al.*¹⁸; and e, water depth at the dredge stations. Note low temperatures at fracture zones, and the two well defined minima south of the Azores and Iceland plateau. Abbreviation for fracture zones (FZ) are: JM, Jan Mayen; S, Spar; T, Tjornes; CG, Charlie-Gibbs; K, Kurchatov; EA, East Azores; O, Oceanographer; and A, Atlantis.

The most notable feature of the temperature profile along the Mid-Atlantic Ridge is the tendency of significantly lower temperatures at or near fracture zones. However, three possible minima are suggested by single dredges only; the Jan Mayen Fracture Zone (1,078 °C), the Gibbs Fracture Zone (1,122 °C), and possibly the 43° N fracture zone (1,131 °C). Although the existing sampling density along the ridge prevents us demonstrating statistically that lavas erupted at or near fracture zones are systematically lower, this trend is sufficient to warrant further discussion and testing. Furthermore, the lower olivine crystallisation temperatures observed for fracture zone basalts are in keeping with the occurrence of more evolved basalts in other Mid-Atlantic Ridge transform fault zones, most probably as a result of fractional crystallisation²². Some variation in the extent of partial melting along the ridge is also likely. Shallow depth fractional crystallisation may affect significantly the Mg value of magmas but has only a minor effect on the La/Sm ratio^{21,23,25} (Fig. 3). On the other hand La/Sm is more and the Mg value less susceptible to variation in extent of partial melting^{24,25}. Some local correlation may exist between La/Sm and the Mg value or the temperature of olivine crystallisation as a result of the combination of these two processes. However, the large amplitude and wavelength variations in La/Sm shown in Fig. 3 are primarily reflections of mantle source heterogeneities in this ratio beneath the ridge and clearly do not parallel the Mg value^{21,25}.

Two pronounced and statistically better defined thermal minima are also observed on the extension of the Reykjanes Ridge over the southern edge of the Iceland Plateau (~63°10'N) and the ridge transect over the southern part of the Azores Platform (~38°30'N) (Fig. 3). The temperature minimum south-west of Iceland first observed by Hermes and Schilling¹² was attributed to shallow-level fractional crystallisation. The presence of another well-defined minimum over the southern part of the Azores Plateau¹³, again another hotspot, suggests the possibility of a common cause for such phenomena. Both minima share the following characteristics: (1) they occur on or over the flank of two major volcanic plateaus or topographic bulges; (2) they are probably underlain by a thicker crust than normally present along ridge segments further remote from the Azores or Iceland^{3,5,26}; (3) the axis of these two ridge segments is displaced in en-echelon fashion by a series of small fracture zones^{5,21}; (4) magnetic anomaly lineaments typifying ridge axes have lost coherency along these two ridge transects^{5,27}; (5) complex recurrent rift jumping has also been suspected in these two regions²⁸; (6) both ridge segments are geochemical transition zones characterised by large-scale radiogenic isotope and La/Sm gradients (Fig. 3) which have been interpreted as reflecting preferential flow and mixing beneath the ridge axis of mantle-plume derived with LILE-derived material^{1,21}; (7) basalts erupted in these two regions are more evolved, have lower Mg values and contain larger proportions of phenocrysts than other MAR basalts, including particularly clinopyroxenes (Fig. 3; and Schilling *et al.* in preparation); and (8) basaltic glasses defining these two temperature minima also contain two populations of olivine and plagioclase phenocrysts. Macrophenocrysts have common resorption features suggestive of magma mixing¹⁴, whereas late generation microphenocrysts do not.

Origin of thermal minima near the Azores and Iceland hotspots

Models describing the cooling of the crust-lithosphere along normal ridge segments, where volcanism occurs passively in response to plate divergence, have been treated primarily as a two-dimensional thermal problem in a plane perpendicular to a semi-infinite ridge axis²⁹⁻³². We suggest that on or near 'hotspots' where subaxial and additional tectonic complication seem to occur^{1,8,21}, and across fracture zones, lithospheric cooling must be treated as a three-dimensional thermal problem. More efficient cooling in ridge segments cross cut by fracture

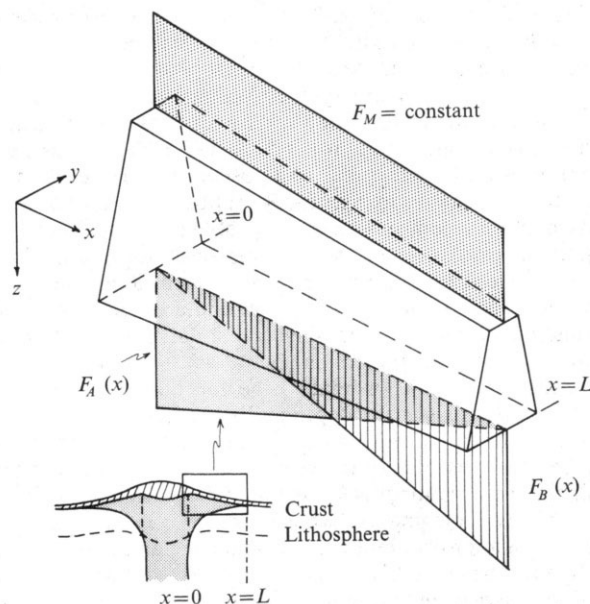


Fig. 4 Model for flow and mixing of magma in subaxial magma chamber. Flux variations with x of the two magma types entering the magma chamber are shown as shaded areas: Grey shading indicates plume-derived magma type A; vertical strips indicate magma type B derived from the LILE-depleted asthenosphere; and dotted area show flux variation with x of mix-magma type M leaving the magma chamber and solidifying as new oceanic crust. Magma flow directions are not shown. Magma type A may enter partly from below or from the end face at $x=0$ and flow along x . Magma type B probably enters mostly from below; whereas mix-magma M partly leaves the magma chamber from its top during dyke injection, or solidifies along both sides due to lateral crustal spreading in the y direction.

zones is to be expected as the active ridge segment abuts against colder lithosphere across the transform fault. More extensive fracturing should also enhance circulation of seawater and heat exchange with magma chambers beneath such regions³³. It has been suggested that the flux of 'mantle plume' material located beneath a ridge is largely independent of spreading rate of the plates above³⁴. As a result, when the flux of mantle plume material per unit ridge length exceeds the rate of plate divergence by seafloor spreading above the plume, the excess material derived from the plume is accommodated in several possible ways: (1) by formation of an elevated ridge axis (rather than a rift valley) along the ridge branching from the plume; (2) stagnation and cooling of magma in unusually large magma chambers at the base of the crust; (3) development of a thicker crust above and about the plume^{1,21}; and (4) lateral flow of magma derived from the plume, subaxial to the ridge, which mixes with complementary melts derived from the LILE-depleted asthenosphere^{1,8,21,35,36}. Modelling and sorting of these different effects are difficult with our present knowledge. An attempt to evaluate the thermal effect of magma flowing and mixing beneath the ridge axis about these two hotspots is made in the following section.

Several mechanisms have been suggested for flow of magma along rifted crustal zones. Vogt³⁵ has modelled sub-crustal and axial pipe-like flow down-slope about hotspot bulges on the basis of gravity and topographic gradients. Fiske and Jackson³⁷, Schilling¹ and Sleep and Rosendahl³⁸ have considered dyke propagation due to non-isostatically compensated topography which causes horizontal deviatoric tension. Direct evidence for intra-crustal flow of magma over 70–100 km distance within Iceland has been provided by Sigurdsson and Sparks³⁹. Whatever the exact cause and nature of the transport mechanism, progressive cooling and crystallisation of the magma is to be expected during flow along the rifted zone. En echelon ridge axis displacements or recurrent rift jumping near hotspots will further complicate the nature of magma plumbing and enhance

controls need to be invoked to explain the shape of these two minima. These include: (1) close-spaced fracture zone fabric near hotspots exposing magma to colder fault planes and further enhancing circulation of seawater within the crust and thus the efficiency of heat loss from the subaxial magma chamber, or (2) lack of a steady-state regime in the subaxial magma chamber beneath these two ridge segments, perhaps signalling recent rift jumping, or tectonic readjustment; or perhaps a simpler explanation (3) the two large temperature minima may reflect the temperature and composition of melts within two large subaxial magma chambers associated with the Iceland and Azores Plateaus.

We consider the cooling effects caused by close-spaced fracture zone fabric or the presence of a large magma chamber as the most probably dominant factors in producing the observed temperature minima south of Iceland and the Azores, as our empirical model alone, involving cooling by subaxial flow and

mixing, provides too broad a minimum. However, this does not mean that subaxial flow and mixing does not occur, as it is also suggested by previous petrographic and geochemical evidence. We also suggest that the lower quenching temperatures of mid-ocean ridge basalts erupted near major fracture zones as compared with magma erupted along unfractured ridge segments may be caused by exposing magma chamber to colder fault planes due to seafloor spreading and by enhancing seawater circulation. The study of basalts collected at close intervals near other fracture zones and hotspots coupled with detailed state-of-the-art geophysical surveys should help in testing our inferences further.

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Mesozoic ophiolite and blue amphibole on Timor and the dispersal of eastern Gondwanaland

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A dismembered metamorphosed ophiolite on Timor is connected with Jurassic rifting of Australia's northwestern margin. The rift developed into an ocean basin which carried a microcontinental block northwards. During the middle to late Cretaceous the block accreted to South-east Asia and experienced a low grade metamorphism which generated crossitic amphibole.

It is generally accepted that Timor is located in a collision zone between South-east Asia and the Australian continent, which separated from Gondwanaland during the Mesozoic. However, the place of origin of the different formations and tectonic units of Timor has been widely debated. Three principal models have been proposed that account for the tectonic development of Timor in radically different ways. Hence, its structure is regarded either as an imbricate subduction mélange of Australian components (the imbricate model)¹, a series of block-faulted Australian formations with surficial gravity slides (the autochthon model)², or as a number of overthrust South-east Asian units on para-autochthonous Australian rocks (the overthrust model)³.

These interpretations have not given adequate consideration to the importance of radiolarian cherts, a suite of metamorphosed igneous rocks, and reports of blue amphibole on Timor:

these key elements can unravel the tectonic history of the island. A reappraisal of such data is used here to develop a new model.

Stratigraphic units and their relationships

The Lolotoi Unit is one of the main structural components of Timor. It comprises a diverse assemblage of ?Precambrian to Miocene age rocks³. The unit includes the Palelo Group and the Lolotoi Complex. The latter replaces and combines two litho-stratigraphic groups—the crystalline schists and ophiolite-spillite complex (Fig. 1). These informal terms are used here.

The crystalline schists are divisible into a low grade younger component and a higher grade older component⁴⁻⁶. The older element is polymetamorphic and dominated by primary amphibolite facies assemblages. Semi-pelitic gneisses can contain a kyanite-staurolite-garnet paragenesis⁷, typical of intermediate pressure and temperature conditions of regional metamorphism. The monometamorphic younger component is characterised by greenschist facies and lower grade mineral assemblages. Relict clastic and igneous minerals and textures are frequently preserved in the least altered rocks⁴. Typical lithologies include Mn-rich metachert, greenstone (such as spilite) and sericitic phyllite. The older component shows abundant evidence of widespread greenschist facies retrogression⁸, possibly associated with the metamorphism which affected the

low grade rocks⁶. In several areas of Western Timor the crystalline schists are overlain unconformably by the Palelo Group (Fig. 1). This comprises a lower series which contains radiolarian cherts and altered basaltic rocks, and an upper part (the Haulasi Formation) dominated by flysch-type deposits.

There is sufficient field and petrographic evidence to demonstrate that the low grade crystalline schists of the Miomaffo district of Western Timor⁴ are closely related to the lower Palelo, and that the ophiolite–spilite complex is broadly coeval with both. Spilite and keratophyre occur in the lower Palelo and low grade component of the Lolotoi Complex. Volcanic breccias near the base of the lower Palelo contain clasts of metamorphosed and strongly deformed basalts, presumably eroded from the low grade rocks, together with less-deformed and metamorphosed basalt clasts derived from interlayered flows. The lower Palelo itself is weakly metamorphosed and often strongly deformed⁴. Igneous activity and deposition were evidently broadly contemporaneous with, but outlasted by, metamorphism and deformation.

Cherts of the lower Palelo contain radiolaria of late Jurassic to early Cretaceous type, while limestones near the top of the sequence contain foraminifera of Aptian to Turonian age⁹. The occurrence of apparently related igneous rocks within the lower Palelo, ophiolite–spilite complex and low grade crystalline schists implies a Jurassic to Cretaceous age for the entire section.

The ophiolite–spilite complex includes gabbroic rocks and extensive masses of ultramafics, which show varying degrees of deformation and metamorphism. Published evidence suggests that serpentinite underlies the crystalline schists in the Mutis^{7,10} and Miomaffo⁴ districts. In other areas, however, serpentinite is intercalated with, or rests on top of, the schists^{8,11}. All cases lack a high temperature thermal aureole and the ultramafics are generally strongly deformed. The ophiolite–spilite complex is older than the unmetamorphosed Haulasi Formation, in which clasts of the complex are found⁷. The tectono-metamorphic episode which affected the complex and the lower Palelo is thus bracketed between the Upper Jurassic and Upper Cretaceous.

The Boi Massif of Western Timor is composed of biotite–garnet–gneiss, serpentinite, metagabbro and amphibolite gneiss¹¹. Homogeneous metagabbro is transitional between an interlayered sequence of amphibolite and metagabbro. This unit contains concordant intercalations of high-grade metasedimentary gneiss. Thin sections of the gneiss show sillimanite pseudomorphing biotite, and polygonal aggregates of cordierite and

hercynite replacing biotite, garnet and sillimanite. The mineral assemblages record a temperature increase to around 700 °C and a pressure decrease equivalent to an uplift of over 7 km (ref. 11). I consider that intrusion of a gabbroic mass caused a thermal overprint during uplift of the lower continental crust. The pelitic gneiss was already regionally metamorphosed, but emplacement of the gabbro preceded further metamorphism and deformation. If the gabbro is related to the ophiolite–spilite complex, this provides additional evidence for Jurassic–Cretaceous 'orogenic' events within the Lolotoi Unit.

The Haulasi Formation is a late Cretaceous to Palaeocene flysch sequence which contains clasts derived from the lower Palelo, crystalline schists and ophiolite–spilite complex^{4,7}. There are also fragments of vesicular basalt which resemble alkaline lavas in the Permian Maubisse Formation of Timor.

The Lolotoi Complex and Palelo Group are intimately related. If they formed part of the northwestern margin of Gondwanaland, it should be possible to correlate their development with the geology of subsurface rocks to the south of Timor, and with Australian shelf and rise deposits which outcrop on the island. Recent marine and on-land investigations have shown that Australian continental crust underlies the Timor Sea¹² and most, if not all, of Timor¹³. Published accounts^{14–16} of the Bonaparte Gulf and Wharton basins (Fig. 2) are used as a basis for the following discussion.

Pre-middle Jurassic correlation

It is generally agreed that the northwestern Australian shelf became fragmented by rifting and major uplift in the late Carboniferous. This exposed Precambrian rocks, on which were deposited late Carboniferous to late Permian sediments. This sequence has been considered as a lateral equivalent of the 'Permian' Aileu and Permian Maubisse formations of Timor¹⁷. The latter contains rift-type alkaline basalts¹⁸ which are similar to those in the Australian facies Cribas Formation of Timor¹⁷. It has been suggested that reefal carbonates of the Maubisse Formation might have developed on a basement high near the northern margin of Australia, this basement being represented by the Lolotoi Complex¹⁷ (probably the older component of the crystalline schists).

In the Bonaparte Gulf Basin, late Carboniferous to early Triassic sedimentation is characterised by a series of marine transgressions and regressions. On Timor, the reported inter-layering of the Maubisse Formation with Australian continental

Brouwer ²⁹	Haile et al ⁹	This article		
Palelo Series	Upper	Haulasi Fm	Upper Cretaceous – Palaeocene	coarse to fine clastics with volcanics
	Lower	Noni Fm	late Jurassic – Upper Cretaceous	limestones and radiolarian cherts
		Metan Fm		radiolarian cherts, clastics
crystalline schists	Lolotoi Complex	crystalline schists	? pre-late Jurassic Mesozoic	spilite, agglomerate
			? pre-Permian	metachert, phyllite, spilite
ophiolite – spilite – complex		ophiolite – spilite – complex	mid-Jurassic to early Cretaceous	schist and gneiss
				metagabbro, serpentinite, tremolite schist

Fig. 1 Nomenclature and stratigraphy of pre-Eocene formations in the Lolotoi Unit.

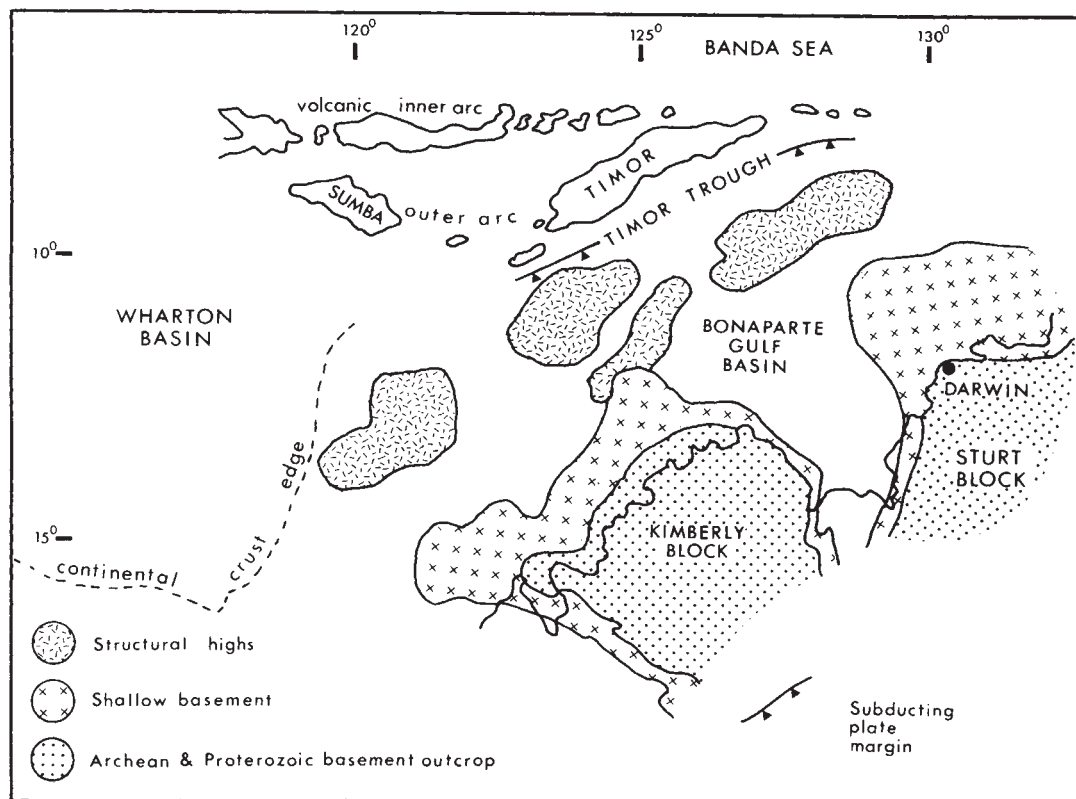


Fig. 2 Location map. Pre-Permian basement ornamented. Modified after Kaman-Kayes and Meyerhoff¹⁹.

rise deposits (Cribas Formation)¹⁷ is explicable in terms of these relative changes in sea level, which must have caused lateral facies changes. If these units intertwine it would prove that the Maubisse Formation was deposited on the northern margin of eastern Gondwanaland, and is not an allochthonous unit derived from South-east Asia as claimed by supporters of the overthrust model³.

Middle Jurassic to middle Cretaceous correlation

In the Bonaparte Gulf Basin middle Jurassic sedimentation was interrupted by a major regional epeirogeny. Precambrian basement became exposed in highs of the horst and graben structures which developed before recommencement of deposition in the Upper Jurassic. Extensive areas of basaltic rocks on the north-west shelf¹⁹ might be related to the formation of new ocean crust (the Indian Ocean)¹⁵ and to uprisen mantle identified under the Bonaparte Gulf Basin²⁰. It can be argued that part of the ophiolite-spilite complex represents middle to late Jurassic igneous activity associated with rifting of the Australian continental margin. Gabbroic magma invaded continental basement which was being uplifted and eroded as part of the rifting process.

In the Bonaparte Gulf Basin, Precambrian blocks supplied detritus until the Neocomian, when all traces of a northerly and northwesterly sediment source were eliminated, presumably by seafloor spreading^{15,16}. The landmass which supplied the sediment has been identified as the submarine ridge which underlies Sumba and the islands towards Timor²⁰ (Fig. 2). Therefore, the rift possibly extended as far as Timor, and perhaps beyond. Rapid subsidence and a marine transgression in the Aptian allowed deposition of bathyal radiolaria- and glauconite-rich sediments in the Timor Sea region¹⁵. They rest uncomfortably on Upper Jurassic to Precambrian rocks, just as bathyal radiolarian cherts of the lower Paleozoic rest uncomfortably on the Lolotoi complex.

Thus far, the autochthon model^{2,17} correlates well with the stratigraphic and structural development of Australia's north-western margin, but during the Cretaceous the two begin to diverge²¹.

Middle Cretaceous to Palaeocene developments

Oceanic crust (the Indian Ocean) formed as a result of rifting which affected the continental crust right down the western side of Australia²². A separate plate, the Wharton Basin (Fig. 2), closed eastwards to a pole of rotation north of Timor¹⁴. This developing ocean basin must have transported rifted continental crust (the Western Landmass) northwards towards South-east Asia. It is inferred that this landmass included the Lolotoi Complex, and perhaps the Aileu and Maubisse formations. Using different criteria Barber²¹ and Johnston²³ have proposed that a microcontinental block separated from Australia in the region of Timor, and accreted to South-east Asia during the Cretaceous.

The Haulasi Formation does not contain clasts derived from the Australian shelf and rise deposits of Timor^{4,5}. This implies that throughout the Upper Cretaceous and Palaeocene the Lolotoi Unit was nowhere near the northern margin of Australia. A significant proportion of the fragments in the Haulasi Formation was derived from andesitic volcanics, and there are interlayered tuffs⁴. The minerals are often unaltered, in contrast with volcanic, schist and chert fragments eroded from the lower Paleozoic and Lolotoi Complex. The andesitic volcanics might therefore be products of middle to late Cretaceous subduction on the southern margin of South-east Asia.

Blue amphibole has been reported from Timor^{4,5,7,18} and neighbouring Leti²⁴. Petrographic descriptions of basaltic rocks from the Permian Maubisse Formation¹⁸ and the ophiolite-spilite complex⁷ show unequivocally the presence of crossite in these rocks. Crossite occurs as rims around, or as parallel intergrowths with, secondary green amphibole and primary pyroxene and brown amphibole. A crossite-bearing greenschist was found as a clast in the Haulasi Formation of the Miomaffo region⁴. If the amphibole has been identified correctly it implies that crossite grew before deposition of the Upper Cretaceous deposits, and after the formation of the ophiolite-spilite complex, which also contains crossite. This metamorphism possibly represents relatively high pressure-intermediate temperature conditions which could have been realised during accretion of the rifted crustal block against the leading margin of

South-east Asia. Subduction might have been short-lived so that isothermal surfaces were not sufficiently depressed to generate typical blueschist facies conditions. The postulated metamorphic episode is broadly contemporaneous with a major orogenic phase which produced greenschists on many other islands of the Outer Banda Arc²⁵. The 'subducted' block was subjected to uplift and erosion, supplying detritus for the Haulasi Formation. This probably formed a prograding wedge of flysch deposited in the arc-trench gap²¹.

Palaeocene to Recent developments

In several massifs of Western Timor, Eocene strata rest unconformably on the Palelo Group and/or the Lolotoi Complex^{4,5,8,26,27}. The unconformable relationships are possibly connected with isostatic adjustments which probably attended cessation of seafloor spreading in the Wharton Basin (about 55 Myr ago¹⁴), and tectonic activity associated with back-arc rifting which generated the Banda Sea²⁸. These events roughly coincide with the final separation of Australia from Antarctica during the Eocene, and with the culmination of a late Mesozoic to early Tertiary orogenic phase identified in the Outer Banda Arc and Eastern Sulawesi²⁵. Presumably subduction must have occurred south of the microcontinent to explain the presence of andesitic volcanics in the Eocene formations^{4,21}. Andesitic volcanics of Eocene age are also found on Sumba, which probably formed part of the same arc.

The Miocene to Recent history of Timor is well documented, but is open to radically different interpretations¹⁻³. The ideas presented here include elements from each of the three main models. Sometime after the Oligocene, Australian continental crust reached the subduction zone. The outcrop trace of the plate boundary is currently located in the axis of the Timor Trough¹ (Fig. 2). The Australian shelf and rise deposits which outcrop on Timor have thus been transferred to the overriding

plate. Near the south coast of Western Timor, some of these deposits (the Kolbano Unit) are imbricated^{3,10}. They form part of an imbricate wedge behind the trench axis, in accordance with the imbricate model¹. Emplacement of overthrust units³ from the north of this zone was caused by underthrusting of the advancing margin of Australia. They include units derived from the postulated microcontinent, as well as an olistostrome. Some of these formations originated from Gondwanaland, but the post-lower to middle Cretaceous sediments and volcanics were deposited on the southern margin of South-east Asia and in the ocean basin to the south.

Conclusions

The Lolotoi Unit contains a dismembered metamorphosed ophiolite which formed during the breakup of Australia's north-west shelf during the Jurassic.

A rifted block of continental crust drifted northwards from Australia, and accreted to South-east Asia during the Cretaceous.

Crossitic amphibole grew during low grade metamorphism within the block.

Subduction south of the block generated andesitic volcanics during the late Mesozoic and early Tertiary.

The block rifted from mainland South-east Asia and met with the northwards drifting Australian continent during the Neogene.

Australian deposits were transferred to the overriding plate and some became imbricated, whereas formations closer to the inner arc were emplaced southwards as overthrust units.

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The transforming gene of Moloney murine sarcoma virus

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A cleavage map of the Moloney murine sarcoma viral DNA was constructed and compared with that of a spontaneously occurring deletion mutant. By restriction enzyme analysis, it was shown that a region encompassing over 40% of the viral information was not essential for transformation or rescue of the deletion mutant. The transforming region was further localised by analysis of the transforming activity in tissue culture of isolated restriction fragments of linear double-stranded sarcoma viral DNA.

In each case, DNA fragments that retained transforming activity preserved the cell-derived insertion sequences of the viral genome. Moreover, such transformants invariably expressed RNA specific to this region. By these two approaches, it was possible to demonstrate that the transforming region of the viral genome begins very near or within the cell-derived insertion sequences. Thus, the transforming gene of this mammalian sarcoma virus originates from within the mouse cell genome.

RNA-CONTAINING sarcoma viruses that transform fibroblasts in tissue culture and induce solid tumours *in vivo* have been isolated from several mammalian species. These replication-defective viruses possess some genetic information of the type-C helper leukaemia virus isolated in association with them¹⁻³. In addition, they contain non-helper viral sequences that are genetically related to cellular DNA of the species from which the sarcoma virus was derived³⁻⁵. By heteroduplex and oligonucleotide mapping, as well as by hybridisation techniques, non-helper viral sequences have been localised to a specific region of the sarcoma viral genome⁶⁻⁸. Thus, accumulating evidence suggests that mammalian sarcoma viruses may have arisen by recombination of a type-C helper virus with some cellular genetic information.

The replication-defective nature of mammalian sarcoma viruses has made genetic analysis of these viruses very difficult. Thus, it has not been possible to identify the region of the viral genome necessary for malignant transformation. Recently, the application of restriction endonuclease technology has led to generation of a physical map of the Moloney strain of murine sarcoma virus (MSV)⁹. In the present report, we have taken advantage of this biochemical approach to identify portions of the viral genome which can be deleted either in a naturally occurring mutant or in a restriction enzyme cleavage product, without affecting the transforming ability of the genome. By this strategy, it has been possible to identify and map directly the transformation-inducing information of MSV. The present findings have implications concerning the origin of transforming genes of mammalian sarcoma viruses.

Comparison of the physical map of a transforming deletion mutant with parental MSV-124 DNA

The major species of viral DNA in NIH/3T3 cells newly infected with the 124 strain¹⁰ of MSV is a linear double-stranded molecule, postulated to be a complete transcript of MSV RNA⁹. Several restriction endonucleases cut this DNA, and a preliminary physical map of the MSV genome has been constructed⁹. Using the superior Southern technique of direct transfer of DNA fragments from agarose gels to nitrocellulose sheets¹¹, we refined and extended this map. Linear MSV-124 DNA was purified from infected cells and digested with several restriction endonucleases. The resulting fragments were fractionated on

agarose gels, blotted onto nitrocellulose sheets, hybridised to ³²P-labelled viral complementary DNA (cDNA) and autoradiographed.

Linear MSV-124 DNA, with a molecular weight (MW) of $3.65 \pm 0.1 \times 10^6$, was cleaved by *XhoI* enzyme (Fig. 1a) into two fragments of MWs 2.4×10^6 and 1.25×10^6 . Digestion with *SalI* endonuclease (Fig. 1b) generated fragments of MWs 1.85×10^6 and 1.75×10^6 . Digestion with *BglII* enzyme (Fig. 1c) produced two fragments of MWs 2.25×10^6 and 1.35×10^6 . *BglI* (Fig. 1d) cut the DNA into fragments of MWs 2.35×10^6 , 1.05×10^6 and 0.25×10^6 . *XbaI* digestion (Fig. 1e) yielded fragments of MWs 2.4×10^6 and 1.25×10^6 . The cleavage products of MSV DNA digested with *HincII*, *HaeII* and *HindIII* enzymes have been reported elsewhere⁹. More accurate determination of the size of the different fragments by the blotting method showed that *HincII* endonuclease cleaved the DNA into fragments of MWs 1.7×10^6 , 1.05×10^6 and 0.85×10^6 ; *HaeII* enzyme cut MSV DNA into three fragments of MWs 2.4×10^6 , 0.95×10^6 and 0.3×10^6 ; finally, *HindIII* enzyme cleaved the DNA into fragments of MWs 3.1×10^6 and 0.55×10^6 .

The cleavage sites of *HindIII* and *HincII* enzymes on MSV-124 DNA were orientated with respect to the 3' and 5' ends of the viral RNA⁹. By a series of double digestions (not shown), it was possible to order the different cleavage sites of the enzymes above into a precise and detailed physical map of MSV DNA (Fig. 2).

We have recently isolated a series of transforming deletion mutants of MSV-124 following serial passage of the parental virus in mouse cells (E.C., K.C.R. and S.A.A., in preparation). Among the mutants analysed, MSV-124 clone 14 was found to have the smallest genome, composed of approximately 3,600 nucleotides. This viral genome was rescued from nonproductively transformed NRK cells by superinfection with a heterologous helper virus, woolly monkey type-C virus. The rescued virus was then used to infect NRK cells so as to obtain unintegrated viral DNA. The linear form of MSV-124 clone 14 viral DNA (Fig. 1f), which has a MW of 2.25×10^6 , was analysed for its susceptibility to those restriction enzymes used for mapping the parental viral genome.

XhoI, *SalI* and *BglII* enzymes (Fig. 1g, h, i respectively) did not cleave MSV-124 clone 14 DNA, indicating the lack of cleavage sites for these enzymes in the mutant viral DNA. The *HaeII* enzyme (Fig. 1l) produced fragments of MWs 1.30×10^6 and 0.95×10^6 , whereas *BglI* digestion (Fig. 1j) generated two

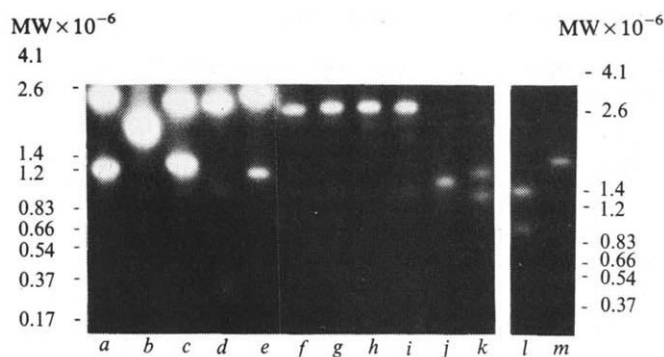


Fig. 1 Analysis of intact and endonuclease-digested MSV-124 and MSV-124 clone 14 DNAs. Linear MSV DNA was obtained from NIH/3T3 cells infected with the 124 strain of MSV or from NRK cells infected with MSV-124 clone 14. About 14 h after infection Hirt supernatant DNA was prepared by a modification of the Hirt procedure²⁸, and linear MSV DNA was further purified by sucrose gradient centrifugation⁹. Aliquots (2-μg) of MSV-124 DNA corresponding to the amount extracted from 5×10^6 cells, were incubated with endonucleases *XhoI*, *SalI*, *BglII*, *BglI* and *XbaI* (lanes a-e, respectively). Similarly, 10-μg aliquots of MSV-124 clone 14 DNA corresponding to the amount extracted from 20×10^6 cells were sham treated (f) or incubated with enzymes *XhoI*, *SalI*, *BglII*, *BglI*, *XbaI*, *HaeII* and *HindIII* (lanes g-m, respectively). Digestion buffers for *HindIII* and *HaeII* enzymes have been reported elsewhere⁹. *BglI* and *BglII* reaction mixtures contained 6 mM NaCl, 8 mM MgAc, 10 mM Tris-HCl, pH 7.5, 6 mM KCl (60 mM KCl for *BglI*), 2 mM dithiothreitol and 100 μg ml⁻¹ bovine serum albumin (BSA). Reaction mixtures for *SalI*, *XhoI* and *XbaI* digestions included 140 mM NaCl, 10 mM Tris-HCl, pH 7.9, 10 mM MgAc, 2 mM dithiothreitol and 100 μg ml⁻¹ BSA. Digestions were carried out in

0.1 ml with the appropriate amounts of each enzyme (New England Biolabs). Following incubation for 2 h at 37 °C, DNAs were extracted with phenol, concentrated by alcohol precipitation, redissolved in 0.05 ml of 10 mM Tris-HCl, pH 7.5, and 1 mM EDTA and loaded on a horizontal slab gel of 1.4% agarose. Reference DNA markers of *HindIII*-digested λ phage DNA²⁹ and *HaeIII*-digested ΦX174 phage DNA³⁰ were electrophoresed in parallel. The reference DNA markers at the left side apply to lanes a-k; those at the right side apply to lanes l, m. Electrophoresis was for 15 h at 30 V and 25 mA in a buffer containing 40 mM Tris-HCl, pH 7.7, 5 mM NaAc and 1 mM EDTA. The DNA was blotted from the gel and fixed onto nitrocellulose filters as described elsewhere³¹. The filters were incubated at 65 °C for 2 h with a solution of 3 × SSC, 0.02% BSA, 0.02% Ficoll and 0.02% polyvinylpyrrolidone, and further incubated at 65 °C for 2 h with the same solution supplemented with sheared native and denatured calf thymus DNA (50 μg ml⁻¹ each), 0.1% SDS and 1 mM EDTA. The nitrocellulose sheet was then transferred to a hybridisation buffer containing 10⁶ c.p.m. ml⁻¹ of ³²P-labelled MSV(MuLV) cDNA. (This viral probe was prepared by reverse transcription of 30S MSV(MuLV)-124 RNA, primed with calf thymus DNA oligonucleotides. The reaction mixture included 20 mM Tris-HCl, pH 7.5, 20 mM KCl, 6 mM MgAc, 1 mM dithiothreitol, 18 μg ml⁻¹ actinomycin D, 1 mM each of dATP, dGTP and dTTP, 0.5 mM ³²P-labelled dCTP at a specific activity of 2-3,000 Ci mmol⁻¹ (Amersham/Searle), 10 μg ml⁻¹ viral RNA, 1 mg ml⁻¹ calf thymus DNA oligonucleotides³² and purified avian myeloblastosis viral reverse transcriptase. Following incubation for 3 h at 37 °C, the cDNA was deproteinised and treated with alkaline to remove viral RNA.) Following hybridisation at 65 °C for 40 h, the strips were washed for 1 h at 65 °C with five changes of hybridisation buffer without cDNA and then washed for 30 min at 65 °C with a solution of 0.1 SSC, 0.1% SDS. The filters were then dipped in 3 mM Tris at pH 9.3 at 4 °C and finally rinsed in 1 M NH₄Ac and dried. Strips were mounted on Whatman paper and subjected to autoradiography (Kodak X-Omat R film) for 24 h at room temperature. The small fragments of MN 0.25×10^6 and 0.55×10^6 in lanes d and m are difficult to visualise in this figure.

fragments of MW 1.1×10^6 , which were indistinguishable in their electrophoretic mobility. These results indicated that the DNA of the deletion mutant retained only one of the two cleavage sites for both *HaeII* and *BglI*. Finally, the DNA was cleaved by *XbaI* (Fig. 1k) to fragments of MWs 1.25×10^6 and 1.0×10^6 , and by *HindIII* (Fig. 1m) to yield fragments of MWs 1.7×10^6 and 0.55×10^6 . Therefore, *XbaI* and *HindIII* sites were retained in MSV-124 clone 14 DNA. These series of digestions enabled us to construct the physical map of the deletion mutant and to define precisely the portion of MSV-124 DNA deleted during generation of the mutant (Fig. 2). This segment of 2,200 nucleotides resided between, but did not include, the *XbaI* and the 5'-terminal *HaeII* sites of MSV-124 DNA. The deletion mutant was efficiently rescued and possessed transforming activity of high titre, indicating that the deleted segment of the MSV-124 genome is not required for virus replication or cell transformation.

Identification of infectious MSV DNA

An independent approach for defining the region of the viral genome required for cell transformation involved the use of intact and fragmented viral DNA in transfection assays. First, we investigated whether *in vivo* synthesised MSV-124 DNA possessed biological activity. Low molecular weight DNA extracted from cells infected 14 h earlier with MSV (MuLV) was fractionated by agarose gel electrophoresis. The DNA was eluted from the gel slices and tested for focus-inducing activity by a modification of the Graham and von der Eb transfection method¹². The transforming activity migrated as a single peak with a mobility slightly greater than that of a 4.1×10^6 -MW linear marker DNA (Fig. 3). Thus, the focus-inducing activity in the Hirt supernatant consisted primarily of unintegrated viral DNA in the form of a linear double-stranded molecule of MW $3.7 \pm 0.2 \times 10^6$. We also analysed the Hirt supernatant DNA, fractionated by agarose gel electrophoresis, for the presence of infectious MuLV DNA. A single peak of reverse transcriptase-inducing activity was detected in the gel at a position of linear double-stranded DNA of MW $5.9 \pm 0.2 \times 10^6$ (Fig. 3), corresponding to the known MW of MuLV DNA^{13,14}.

Transformation by MSV-124 DNA restriction fragments

To establish the infectivity of DNA fragments and, thus, to localise further the region of the MSV genome responsible for

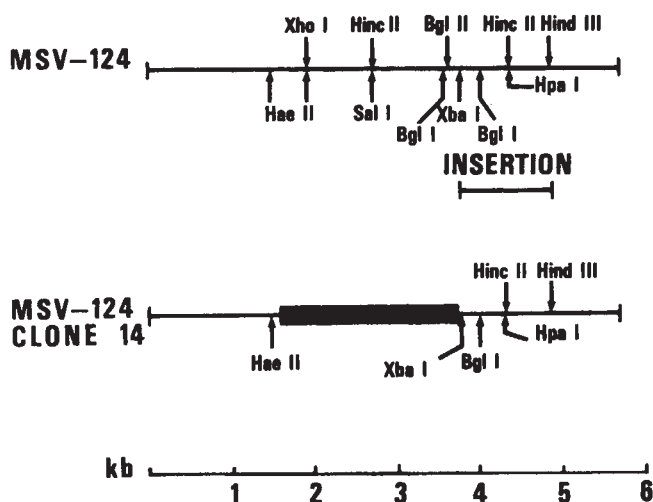


Fig. 2 Physical map of MSV-124 and MSV-124 clone 14 linear DNAs. The right-hand side represents the DNA region corresponding to the 3' terminus of MSV RNA. The position of *HpaI* cleavage site has been reported elsewhere²². The initial study on the topography of the cellular insertion in the MSV genome was made by Hu *et al.*⁶. The location and size of the insertion as shown here are based on more accurate recent data of G. Vande Woude and colleagues (personal communication) as well as our own results (Tronick *et al.*, in preparation).

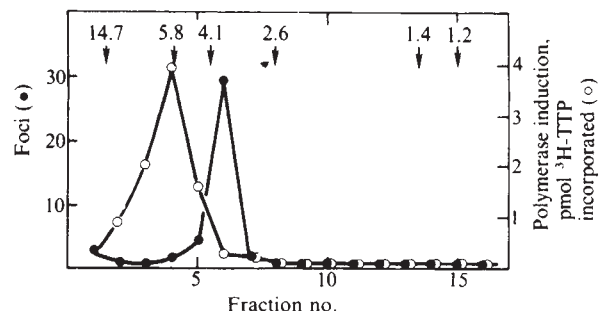


Fig. 3 Agarose gel electrophoresis of infectious MSV and MuLV DNA. Hirt supernatant DNA was prepared from cells infected with MSV-124 as described in Fig. 1. A 35- μ g DNA aliquot, corresponding to that extracted from 3×10^7 infected cells, was fractionated by agarose gel electrophoresis as described in Fig. 1. DNA markers of *HindIII*-digested λ phage DNA were electrophoresed in parallel and are represented as MW $\times 10^{-6}$. The DNA was extracted as described elsewhere²³ from 3-mm gel segments and used to transfect NIH/3T3 cells by modification of the Graham and van der Eb method¹². Following sterilisation with alcohol, the DNA was redissolved in 0.05 ml of $0.1 \times$ SSC and 0.4 ml HEPES buffer, mixed with 25 μ g of sheared calf thymus DNA and precipitated by addition of 2 M CaCl_2 to a final concentration of 125 mM. After 30 min, the precipitate (0.5 ml) was added to 100-mm Petri dish cultures of NIH/3T3 cells, seeded the previous day with 1×10^6 cells. After 20 min, 10 ml of growth medium were added and the cultures were incubated for an additional 3 h. Dishes were then rinsed twice with growth medium and exposed for 4 min to 5 ml of 15% glycerol in HEPES buffer (N. G. Copeland and G. M. Cooper, personal communication). Finally, the cultures were washed with growth medium and incubated for 10 d, at which time they were scored for focus formation and for release of virion-associated reverse transcriptase activity³³.

transformation, MSV DNA was digested with *XhoI*, *BglII*, *HpaI* or *HindIII* enzymes, and fractionated by agarose gel electrophoresis. DNA fragments were then extracted from the gel, and tested by transfection for transforming activity. The fragments were also subjected to agarose gel electrophoresis and blotting analysis to determine their purity. As shown in Table 1, much of the transforming activity of intact linear MSV-124 was retained after digestion with either *XhoI* or *BglII* enzymes. The focus-inducing ability was associated with the *XhoIA* and *BglIIB* DNA fragments, respectively (Table 1). Digestion of MSV-124 DNA with two enzymes, *HpaI* and *HindIII*, which cut within the cellular insertion of MSV-124, abolished the transforming activity. As shown in Table 1, no focus-inducing ability was retained by either *HindIIIA* or *HindIIIB* fragments. Similarly, neither *HpaIA* nor *HpaIB* fragments retained biological activity.

Expression of sarcoma viral information in cells transformed by MSV DNA fragments

Transformed foci induced by *XhoIA* or *BglIIB* MSV DNA fragments were isolated by the cloning cylinder technique, and several such focus-derived lines were grown to mass culture for further analysis. None of several individual transformants induced by each of the fragments released detectable levels of reverse transcriptase. These findings were not unexpected in view of the fact that these restriction enzymes inactivated the infectivity of helper viral DNA. By morphological criteria, cells transformed by either viral DNA fragment were indistinguishable from transformants induced by intact virus or linear viral DNA. The transformants consisted of round or spindle-shaped, highly refractile cells that grew to saturation densities ranging from 3 to 6×10^5 cells cm^{-2} , compared with 6×10^4 cells cm^{-2} for uninfected NIH/3T3 cells. Moreover, they formed colonies at high efficiency when suspended in soft agar and were tumorigenic in NIH/Swiss mice.

We next investigated cells transformed by individual MSV DNA fragments for expression of sarcoma viral RNA. *XhoIA* and *BglIIB* DNA fragments shared a segment of the viral genome, which included the region of MSV RNA previously shown to be MSV specific, that is, not shared with MuLV RNA⁶.

Table 1 Transformation of NIH/3T3 cells by MSV DNA restriction fragments

MSV DNA	DNA extending (kilobase pairs)	DNA added (μ g per plate)	Expt 1	Foci per plate Expt 2	Expt 3
Intact linear form	0-5.7	30 15	55 34	45 ND	65 ND
Restriction fragment*					
<i>Xho</i> IA	1.9-5.7	40	ND	25	38
B	0-1.9	40	ND	0	0
<i>Bgl</i> IIA	0-3.6	40	0	0	0
B	3.6-5.7	40	11	32	43
<i>Hpa</i> IA	0-4.3	40	ND	0	0
B	4.3-5.7	40	ND	0	0
<i>Hind</i> IIIA	0-4.85	40	ND	0	0
B	4.85-5.7	40	ND	0	0

Transfections were carried out in 100-mm Petri dishes and focus formation assayed as described in Fig. 3 legend. ND, Not determined.

* 40 μ g of linear viral DNA was digested with restriction endonucleases, deproteinised, precipitated with alcohol and fractionated by agarose gel electrophoresis. Extraction from gel segments was as previously reported²⁵. The recovery of the different DNA fragments from the gel was comparable as determined by blotting analysis.

Thus, we reasoned that an MSV-specific cDNA probe could be used to detect transcription of *Xho*IA and *Bgl*IIA DNA. Such a probe was prepared from a DNA transcript of MSV(MuLV) RNA by a series of preparative hybridisations. This involved selection for MSV sequences by hybridisation with MSV(FeLV) RNA followed by selection against sequences annealing to MuLV RNA¹⁵.

To characterise the MSV-specific probe, we determined its ability to anneal to MSV and MuLV sequences, respectively. As shown in Fig. 4a, RNA of NIH/3T3 or NRK cells nonproductively transformed by MSV-124 hybridised the entire MSV-specific cDNA, whereas RNA of NIH/3T3 or NRK cells infected with MuLV annealed no more than 6% of the same probe. Further, by analysis of hybridisation of the probe to different MSV DNA fragments (not shown), it was possible to conclude that the MSV-specific cDNA was composed predominantly of sequences included in the region of the genome containing non-helper viral sequences⁶. Finally, the cellular origin of the sequences in the MSV specific cDNA^{3,15} was shown by the ability of normal NIH/Swiss embryo cellular DNA to anneal the probe to a large extent with kinetics reflecting single copy representation (Fig. 4b).

Cellular RNAs of transformants induced by MSV DNA fragments were next tested for their ability to anneal the MSV-specific cDNA probe. As shown in Fig. 5, RNAs of representative *Xho*IA and *Bgl*IIA transformants hybridised more than 40% and 60% of the probe, respectively, at C_{ot} values above 7×10^3 . Similar results were obtained with each of several individual transformants induced by these two DNA fragments. In contrast, RNA of neither NIH/3T3 cells nor NIH/3T3 cells transformed by another murine sarcoma virus, Kirsten MSV, contained detectable amounts of RNA complementary to the MSV-specific cDNA (Fig. 5). These results establish that cells scored as transformed following transfection with MSV DNA fragments expressed MSV-specific RNA.

Comparison of transformants induced by MSV DNA fragments and intact viral DNA

It is well established that replication-defective mammalian sarcoma viruses can be rescued from nonproductively transformed cells by a type-C helper virus¹⁶⁻¹⁸. It was of interest to determine whether focus-forming activity could be rescued from cells transformed by MSV DNA restriction fragments. Following MuLV superinfection, none of several individual transformants induced by *Xho*IA or *Bgl*IIA fragments released detectable focus-forming virus (Table 2). In the same conditions, high-titre MSV was released by cells transformed by intact

MSV-124 or MSV-124 clone 14 virus, or by linear MSV-124 DNA. The amounts of helper virus produced were comparable for all cell lines tested. These results established that only those transformants induced by the MSV DNA fragments lacked rescuable focus-forming activity.

Some MSV strains code for helper virus *gag* gene products^{1,19,20}. By analogy with the avian type-C viral genome²¹ the *gag* gene of the mammalian type-C virus is presumed to be localised to the 5' region of the viral genome. By heteroduplex mapping, MSV has been shown to contain a long region of helper viral sequence homology beginning at the 5' end and extending approximately 2.25 kilobases⁶. Thus, it is likely that this region contains the helper viral sequences coding for *gag* gene proteins.

To determine whether *gag* gene expression might serve as a useful marker of the intact MSV 124 genome, we analysed several MSV 124 and MSV-124 clone 14 nonproducer transformants for expression of the N-terminal *gag* gene protein, p15 (ref. 19). As shown in Table 2, p 15 was detected at levels of 30-500 ng per mg cellular protein in each virus nonproducer transformant analysed. Similarly, nonproducer transformants induced by linear MSV DNA expressed p15. As the *Bgl*IIA DNA and *Xho*IA fragments originate about 3.6 and 1.9 kilobase pairs from the 5' terminus of the MSV genome, respectively, it was considered unlikely that transformants induced by these fragments would possess the coding capacity for MuLV p15. As shown in Table 2, none of the transformants induced by *Bgl*IIA or *Xho*IA DNA expressed detectable levels of this virus-coded protein. These findings are consistent with the assignment of transforming activity to *Xho*I and to *Bgl*II DNA fragments other than those encompassing the 5' terminus of the transforming virus genome.

The transforming gene of mouse sarcoma virus

In the present studies, two independent approaches have been used to localise the region of the Moloney MSV genome

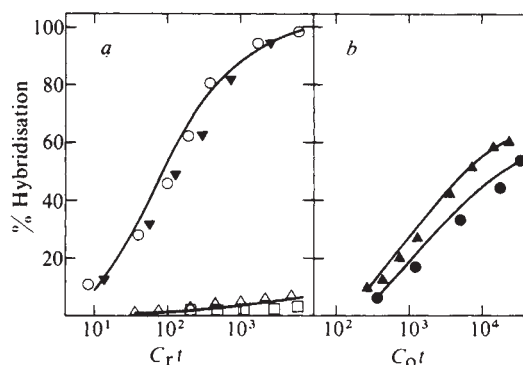


Fig. 4 Characterisation of MSV specific cDNA probe. MSV (MuLV) cDNA was prepared as described in Fig. 1 legend. MSV-specific cDNA was obtained from it by a series of preparative hybridisations in which cDNA sequences unique to MSV were selected by hybridisation with MSV(FeLV) RNA, followed by hydroxyapatite chromatography. Sequences in the cDNA shared with MuLV were removed by two cycles of hydroxyapatite after annealing with MuLV RNA¹⁵. Cellular RNA was purified by the method of Scherrer³⁴. Cellular DNA was prepared as described elsewhere³⁵, and sheared to a mean size of 6-8S. Unique sequence cellular DNA was similarly prepared and highly reiterated sequences annealing at a C_{ot} of 50 mol l⁻¹ were removed by fractionation on hydroxyapatite. For cDNA-RNA hybridisation, varying amounts of RNA were annealed to 500 c.p.m. of ³²P-labelled MSV-specific cDNA in 0.05 ml hybridisation mixtures containing 10 mM Tris-HCl, pH 7.5, 1 mM EDTA and 0.6 M NaCl. After incubation for 18-48 h at 68 °C, hybrid formation was determined with S₁ nuclease. cDNA-DNA annealing and hybrid detection were carried out in similar conditions except that the RNA was substituted with cellular DNA at 4 mg ml⁻¹, and the hybridisation was carried out for varying times at 62 °C. *a*, Hybridisation of MSV-specific cDNA by cellular RNAs of MSV-124 transformed nonproducer NIH/3T3 (○); MSV-124-transformed non-producer NRK (△); Moloney MuLV-infected NIH/3T3 (▽); Moloney MuLV-infected NRK (□). *b*, Hybridisation of MSV-specific cDNA by NIH Swiss mouse embryo DNA (●). The rate of this reaction was compared with the rate of hybridisation of tracer amounts of mouse unique sequence cell ³H-DNA by its homologous unlabelled cell DNA (▲).

involved in transformation. By comparison of the restriction endonuclease map of the viral genome with that of a deletion mutant isolated from the Moloney MSV stock following several cycles of virus passage, a large region encompassing almost 40% of the viral information was shown to be nonessential for transformation or virus replication. The dispensable sequences extend 1.5–3.7 kilobase pairs on MSV-124 DNA. Analysis of the transforming activity of subgenomic DNA fragments isolated following restriction endonuclease cleavage of linear viral DNA revealed that the *Xho*IA and the *Bgl*II B DNA fragments were highly active. The *Bgl*II B fragment, which is the smaller of the two, extends 3.6–5.7 kilobase pairs and therefore lacks not only the 5' terminus, but also about two-thirds of the viral genome. Thus, the evidence obtained by these two approaches fixes the 5' extent of the maximum information necessary for transformation at or near the *Xba*I site, about 3.7 kilobase pairs from the 5' terminus.

In contrast to our findings that most of the transforming activity of the viral DNA was preserved after cleavage with *Xho*I or *Bgl*II enzymes, transforming activity was abolished after digestion with *Hpa*I or *Hind*III enzymes. As the first enzyme cleaves in the middle and the latter cuts near the 3' terminus of the cellular insertion sequences, these findings suggest that the information within a large portion of the cellular insertion are essential for transformation.

Andersson *et al.*²² have recently shown that subgenomic fragments of *in vitro* synthesised MSV DNA retain transforming activity. Their smallest transforming fragment spanned a region of 2.1 kilobase pairs encompassed by the *Sal*I and *Hind*III restriction sites. This fragment contains 0.9 kilobase pairs of additional genetic information at the 5' end compared with the DNA segment shown here to be required for cell transformation. The preservation of transforming activity following *Hind*III digestion of the viral genome as reported by Andersson *et al.*²² is in contrast to our results. The reasons for this discrepancy are unclear. We have also encountered at low frequency transformants induced by MSV DNA fragments generated by digestion with an enzyme, *Hinc*II, which cuts the viral genome within the insertion sequences. However, when these transformants were analysed, they were found to express viral RNA complementary to portions of the viral genome in addition to the *Hinc*II B transforming fragment (spanning 2.7–4.4 kilobase pairs). We were thus unable to conclude that these transformants were induced by the *Hinc*II B fragment alone.

Table 2 Properties of NIH/3T3 cells transformed by MSV DNA restriction fragment

	Sarcoma virus rescue*		M-MuLV p15 expression†	
	NIH/3T3 cell line	No. positive/ No. tested	Range (FFU ml ⁻¹)	No. positive/ No. tested
Transformed by MSV-124 virus		4/4	10 ^{5.4} –10 ^{5.8}	4/4
MSV-124 clone 14 virus		9/9	10 ^{5.4} –10 ^{5.9}	3/3
Linear DNA		5/5	10 ^{5.6} –10 ^{5.9}	2/2
MSV-124 <i>Bgl</i> II B DNA		0/5	<10 ⁰	0/5
MSV-124 <i>Xho</i> IA DNA		0/2	<10 ⁰	0/2
Uninfected		—	<10 ⁰	—

* Around 10 days after infection of each cell line with 10⁵ XC plaque-forming units (PFU) of MuLV, 0.5 ml of tissue culture fluids were assayed for focus-forming units (FFU)¹ and XC PFU²⁶ on NIH/3T3 cells as previously described.

† Cultures were sonicated for 1 min in the presence of 10 mM Tris-HCl, pH 7.8, containing 200 mM NaCl, 0.05 mM EDTA, and 1% (v/v) Triton X-100. The soluble protein fraction, obtained from the supernatant of a 150,000g centrifugation, was tested at twofold serial dilution in an homologous Moloney-MuLV p15 radioimmunoassay as previously described¹⁹. Levels of viral protein expression were calculated on the basis of (1) the amount of cellular protein, determined according to Lowry *et al.*²⁷, and (2) the sensitivity of the p15 immunoassay.

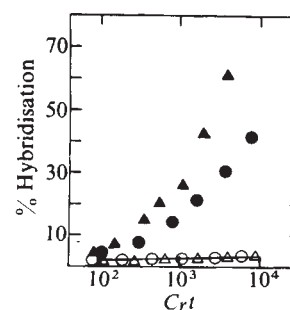


Fig. 5 Expression of MSV-specific RNA in cells transformed by MSV DNA fragments. Hybridisation was carried out as described in Fig. 4 legend. MSV-specific cDNA was hybridised to cellular RNA from uninfected NIH/3T3 cells (Δ), NIH/3T3 cells transformed by the *Bgl*II B MSV DNA fragment ($\blacktriangle$), NIH/3T3 cells transformed by the *Xho*IA MSV DNA fragment ($\bullet$), and NIH/3T3 cells transformed by Kirsten MSV ($\circ$).

Transformants induced by *Xho*IA and *Bgl*II B fragments were shown to express RNA specific to the region of the MSV genome from which the fragments were derived. Moreover, the transformants tested lacked the ability of the parental sarcoma virus to express translational products of the 5' region of the viral genome. The lack of recovery of focus-forming activity from transformants induced by DNA restriction fragments provided even more striking evidence of the phenotypic differences between these cells and transformants induced by virus or intact linear viral DNA. The lack of rescuability of focus-forming activity from transformants caused by MSV DNA fragments remains to be resolved, but may reflect a requirement for preservation of termini of the viral genome for efficient virus rescue and for biological activity of the viral RNA. This possibility is favoured by our findings that the deletion mutant, which lacked a large portion of viral information but preserved both termini, was efficiently rescued and demonstrated transforming activity of high titre.

It has been shown that the genomes of replication-competent type-C viruses of both avian and mammalian origin possess leader sequences originating at their 5' terminus. These leader sequences become associated with messenger RNAs originating from distal portions of the viral genome^{23,24}. Neither of the *Bgl*II B or *Xho*IA MSV DNA fragments with transforming activity contained 5'-terminal sequences of the parental viral genome. It can be argued that MuLV DNA present in the MSV DNA preparations may have acted to complement transformation by MSV DNA. However, we consider this highly unlikely as MuLV DNA represented only a small fraction (5%) of the viral DNA. Moreover, such MuLV DNA would have to co-migrate with each of the different-sized MSV-transforming DNA fragments used for transfection. Presumably, therefore, viral RNAs transcribed from the transforming MSV DNA fragments of *Bgl*II B or *Xho*IA either do not contain leader sequences or derive such a sequence from cellular DNA.

The present studies demonstrate that the helper viral sequences extending from the 5' end of the cellular insert to the 5' end of the MSV genome were not essential for transformation. In contrast, cleavage within the cellular insertion inactivated transforming activity. These findings establish that viral information required for transformation by this mammalian sarcoma virus originates from within the cellular genome. By recombinant DNA techniques, it should be possible to obtain a homogeneous population of MSV DNA molecules for further analysis of these sequences. Such techniques should also allow the isolation, amplification and sequencing of the cellular gene homologous to the transforming region of MSV. These studies may make it possible to determine whether the MSV-transforming gene originates from an altered cellular gene or from one that is normally only expressed before the development of the fully differentiated state.

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letters

Does PSR0329 + 54 have companions?

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An extensive pulse timing programme has been carried out over the past 10 years for several pulsars¹⁻⁵. The timing observations revealed a wide range of different effects such as glitches, noise in the pulsar rotation and unexpectedly large second time derivatives of the rotation frequency. We suggest here that these unexpectedly large values of $\ddot{\nu}$ may be caused by a binary motion with an orbital period, P_b , longer than the span of timing observations. Even a distant planet of a relatively low mass ($100 M_\oplus$ for $P_b = 50$ yr) would influence $\ddot{\nu}$. In the case of PSR0329 + 54, besides a large $\ddot{\nu}$ term, a quasi-sinusoidal modulation shows up in timing residuals. This 3-yr periodicity may be caused by: (1) a change in pulse shape, (2) precession of a magnetic dipole axis, or (3) a small planet ($m = 0.06 - 0.57 M_\oplus$) orbiting the pulsar.

All known pulsars are slowing down at a rate conveniently described by the braking index $n = \nu\ddot{\nu}/\dot{\nu}^2$, where ν is the pulsar frequency. All models of energy loss proposed so far give $n \sim 3$. Using the observed values of ν and $\dot{\nu}$ and assuming $n = 3$ it is easy to estimate $\ddot{\nu}$. It turns out that $\ddot{\nu}$ should be very small (except for the Crab pulsar) and therefore the pulse number at time t can be approximated by

$$N(t) = N_0 + \nu t + \frac{1}{2} \dot{\nu} t^2 \quad (1)$$

However, unexpectedly large values of $\ddot{\nu}$ have been measured for 11 pulsars observed at Arecibo¹, and Helfand *et al.*⁵ have discussed two other pulsars (PSR0611 + 22, PSR0823 + 26) belonging to this class. In addition, the residuals $N_{\text{obs}} - N(t_{\text{obs}})$ shown for PSR0329 + 54 and PSR1508 + 55 (refs 5, 6) also indicate the presence of the large $\ddot{\nu}$ term. Table 1 lists all these

pulsars. It also includes the Crab pulsar, PSR0531 + 21, for which $\ddot{\nu}$ is large because of its very rapid slowdown.

Here we discuss some features of the timing data for PSR0329 + 54. The barycentric arrival times covering 8 yr of pulsar observations⁵ at National Radio Astronomy Observatory and Five College Radio Astronomy Observatory were kindly supplied by Dr D. J. Helfand.

To determine $\ddot{\nu}$ we take

$$N(t) = N_0 + \nu t + \frac{1}{2} \dot{\nu} t^2 + \frac{1}{6} \ddot{\nu} t^3 \quad (2)$$

The values of $\ddot{\nu}$ calculated from data covering the first 5, 6, 7 and whole 8 yr of observations are respectively $\ddot{\nu} \times 10^{25} = 0.66, 0.64, 0.48$ and 0.55 s^{-3} . For the last 5 and 6 yr one gets $\ddot{\nu} \times 10^{25} = 0.94$ and 0.51 s^{-3} . The value of $\ddot{\nu}$ does not depend strongly on the set of data. As our sets of data overlap, this cannot be treated as proof that the effect is a real one. In fact, it has been suggested^{5,7,8} that the observed values of $\ddot{\nu}$ can result from a noise-type variation in the pulsar rotation. A random-walk type phenomenon in the phase, frequency and/or frequency derivative may lead to large values of $\ddot{\nu}$ when the span of timing data has a finite length.

To check the value of $\ddot{\nu}$ and to find whether it is a real effect, it is derived from independent sets of data; noise processes should give a normal distribution around $\ddot{\nu} = 0$. This procedure, however, cannot be applied to PSR0329 + 54 at present. A ratio of the change in the pulsar phase caused by the $\ddot{\nu}$ term after time Δt , $\Delta\phi = \ddot{\nu}(\Delta t)^3/6$, to the measurement uncertainty of the phase determination, $\Delta N = 7 \times 10^{-4}$, is poor for short Δt , hence fits over short spans give meaningless values of $\ddot{\nu}$ because an error assigned is of the order of the fitted value. Moreover a periodic component present in residuals (Fig. 1) strongly influences $\ddot{\nu}$ derived from spans shorter than ~ 5 yr. However, this analysis could be done successfully for pulsars with a larger $\ddot{\nu}$ term such as PSR0823 + 26. Unfortunately, at present we do not have data for other pulsars with large $\ddot{\nu}$.

If the large value of $\ddot{\nu}$ does represent a real property of some pulsars then one also has to accept large and sometimes negative values of the braking index as a real effect. Therefore, a

Table 1 Pulsars with known $\ddot{\nu}$

PSR	Interval fitted (days)	ν (s^{-1})	$\dot{\nu}$ (s^{-2})	$\ddot{\nu}$ (s^{-3})	Braking index*	Refs
0329+54	2,858	1.400	-4.02 E-15	5.55 E-26	(4.81 ± 0.18) E+03	This work
0531+21	1,875	30.206	-3.86 E-10	1.23 E-20	2.515 ± 0.01	4
0540+23	1,367	4.066	-2.55 E-13	3.95 E-25	(2.47 ± 1.02) E+01	1
0611+22	1,584	2.986	-5.31 E-13		3.5 E+02	5
0823+26	1,833	1.884	-6.06 E-15		-1. E+04	5
0950+08	1,562	3.952	-3.58 E-15	-1.70 E-25	(5.26 ± 0.76) E+04	1
1508+55	2,080	1.352	-9.20 E-15		3.25 E+03	6
1541+09	1,668	1.336	-7.68 E-16	-1.12 E-25	-(2.53 ± 0.42) E+05	1
1604-00	1,613	2.371	-1.72 E-15	2.31 E-26	(1.85 ± 0.72) E+04	1
1859+03	1,226	1.526	-1.74 E-14	1.10 E-24	(5.53 ± 0.50) E+03	1
1907+00	780	0.983	-5.33 E-15	-2.49 E-25	-(8.61 ± 1.42) E+03	1
1907+10	993	3.526	-3.28 E-14	-1.69 E-24	-(5.55 ± 0.40) E+03	1
1915+13	1,602	5.138	-1.90 E-13	-6.44 E-25	-(9.15 ± 1.44) E+01	1
1929+10	1,374	4.415	-2.25 E-14	-3.18 E-25	-(2.76 ± 0.26) E+03	1
2002+31	1,606	0.474	-1.67 E-14	7.13 E-26	(1.21 ± 0.90) E+02	1
2020+28	1,637	2.912	-1.61 E-14	1.03 E-25	(1.16 ± 1.80) E+03	1

* An error quoted corresponds to two standard deviations in $\ddot{\nu}$.

theoretical explanation of this phenomenon is urgently needed.

The large value of $\ddot{\nu}$ derived from the data covering a finite span of time could result from: (1) long time scale irregularities of the pulsar rotation, or (2) from external perturbations such as orbital motion of the pulsar. Irregularities of rotation ('glitches') were observed for several pulsars but their characteristic relaxation times were not longer than a few hundred days. In the models of glitches considered so far it is assumed that the irregularities are caused by a sudden change in the moment of inertia of the neutron star^{9,10}. In those models $\ddot{\nu}$ can be large but must be positive during the time of relaxation. Such behaviour was observed for the Vela pulsar after the glitch of September 1975 (ref. 11). For PSR0329+54 the large value of $\ddot{\nu}$ can be explained by this model if the glitch occurred before the discovery of the pulsar and if the relaxation time was longer than the time span of the data. However, it is not possible to say when such a glitch might have occurred. Note that the negative values of $\ddot{\nu}$ observed for some pulsars cannot be explained in this way. Another explanation of this phenomenon is due to Gullahorn and Rankin¹². They suggest the long time scale (10^2 – 10^4 yr) variations in the pulsar braking torque. These variations, caused by varying interaction between pulsar and the medium in its vicinity, might give large values of $\ddot{\nu}$ of both signs.

The orbital motion of a pulsar with an orbital period longer than the span of timing observations is very difficult to detect because the effects caused by this motion are masked by the slowdown of the pulsar¹³. Even if ν and $\dot{\nu}$ are strongly influenced by such a motion their analysis cannot show the presence of a binary system unless $\dot{\nu} > 0$ (refs 13, 14).

This orbital motion can be detected more easily, even for a much lighter companion, by looking at higher derivatives of ν which can be drastically influenced by the orbital motion. Let us consider a pulsar moving on a circular orbit. The distance to the pulsar is $d = d_0 + A \sin(\omega t + \phi_0)$ with

$$A = \left[\frac{G}{\omega^2(M+m)^2} \right]^{1/3} m \sin i,$$

M and m being the mass of the pulsar and its companion respectively. Suppose that during the period of observations $\omega t + \phi_0$ does not change significantly. If $\phi_0 \approx 0$ or π then $d - d_0$ can be approximated by $\pm A(\omega t - \frac{1}{2}\omega^3 t^3)$; if $\phi_0 \approx \pi/2$ or $3\pi/2$ then $d - d_0 \approx \pm A(1 - \frac{1}{2}\omega^2 t^2)$. Thus, in the most favourable configurations the maximal contributions to ν , $\dot{\nu}$ and $\ddot{\nu}$ are given by $\Delta\nu = \pm\nu A\omega/c$, $\Delta\dot{\nu} = \pm\nu A\omega^2/c$, $\Delta\ddot{\nu} = \pm\nu A\omega^3/c$. If, for example, $P_{\text{orb}} = 2\pi/\omega = 50$ yr, $M = 1M_{\odot}$, $\sin i = 1$ then for $m \ll M$ one gets $A = 6.1 \times 10^8 (m/M_{\oplus})$ cm, $\Delta\nu/\nu = \pm 8.1 \times 10^{-11} (m/M_{\oplus}) s^{-1}$, $\Delta\dot{\nu}/\nu = \pm 3.2 \times 10^{-19} (m/M_{\oplus}) s^{-2}$ and $\Delta\ddot{\nu}/\nu = \pm 1.3 \times 10^{-27} (m/M_{\oplus}) s^{-3}$, where $M_{\oplus}$ is the mass of the Earth.

Comparing the above values with those in Table 1 we see that the orbital motion of a pulsar caused by a distant planet of a small mass may give large values of $\ddot{\nu}$ but a negligible change in $\dot{\nu}$ (in comparison with typical slowing down rates of pulsars). This shows that a large value of $\ddot{\nu}$ hints that the pulsar may be accompanied by a distant planet and therefore should be closely watched in the future. Unfortunately, the extensive timing project at Arecibo¹ was discontinued in 1976.

Figure 1 shows the residuals $N_{\text{obs}} - N(t_{\text{obs}})$ for PSR0329+54 with $N(t)$ given by equation (2). There is an apparent periodic component which shows up when we use data covering any period longer than five years. Let us assume that

$$N(t) = N_0 + \nu t + \frac{1}{2}\dot{\nu}t^2 + \frac{1}{6}\ddot{\nu}t^3 + a \sin \frac{2\pi}{P_{\text{orb}}}(t - t_0) \quad (3)$$

Using the least squares procedure one gets $N_0 = -0.352937 \pm 0.000296$, $\nu = 1.3995435677615 \pm 1.32 \times 10^{-11} s^{-1}$, $\dot{\nu} = -4.021220 \times 10^{-15} \pm 2.56 \times 10^{-19} s^{-2}$, $\ddot{\nu} = 5.553 \times 10^{-26} \pm 2.05 \times 10^{-27} s^{-3}$, $a = (1.201 \pm 0.142) \times 10^{-3}$, $P_{\text{orb}} = 1,105.2 \pm 25.2$ d and $t_0 = 518.3 \pm 29.7$ d. All these values refer to the epoch JD 2,440,621.5. The errors assigned to these values are twice the formal standard deviation as derived from the least square fitting process with measurement errors assumed to be 500 μs (ref. 5); $\chi^2 = 315$, the number of degrees of freedom of the fit is $252 - 7 = 245$ and the r.m.s. residual between the fit and the data is 559 μs .

The sinusoidal term corresponds to a displacement with an amplitude of only 257 km over the period of 3 yr! This term

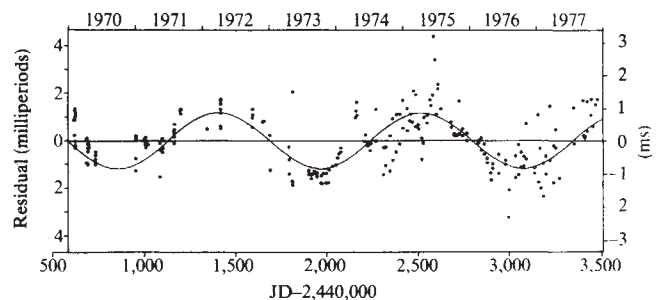


Fig. 1 The residuals for PSR0329+54 after subtraction of a best fitted cubic polynomial. The r.m.s. residual between the fit and the data is 774 μs ; $\chi^2 = 603$ for $252 - 4 = 248$ degrees of freedom. The best fitting sinusoid (see equation (3) of text) is also marked.

could result either from a periodic displacement of the emission region in the pulsar magnetosphere caused by precession of the magnetic axis or a very small (858 μ s) modulation of the pulsar profile which might have passed unnoticed. A small planet around the pulsar could cause the same effect. The mass function derived from the fitted parameters is $5.57 \times 10^{-19} M_{\odot}$. Masses of neutron stars are believed to be in the range of 0.1 – $3 M_{\odot}$ so the lower limits on the mass of a companion ($\sin i = 1$) are correspondingly $0.06 M_{\odot}$ and $0.57 M_{\odot}$.

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VLBI Observations of the double QSO, 0957 + 561 A, B

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Walsh *et al.*¹ originally suggested that the double QSO 0957 + 561 A, B might be images of the same object formed by a gravitational lens because of their remarkably similar optical spectra. Additional optical spectral data² strengthen the evidence for a gravitational lens. Radio data at 5 GHz have shown a complex structure^{3,4}. There are compact (<1 arc s) components closely coincident with the two QSOs; Pooley *et al.*³ suggested that the one near B may be extended ~ 1.5 arc s, but the higher resolution data of Roberts *et al.*⁴ make it probable that B is, in fact, also compact. In addition, there are at least two extended components, one to the north-east producing more than half the total 5-GHz flux density, and a much weaker one to the west. Pooley *et al.* show that these two extended components have fairly steep spectral indices (-0.65) between 5 GHz and 408 MHz but the components close to the QSOs have relatively flat spectra. We have now tested the gravitational lens hypothesis using the greater resolution obtained by very long baseline interferometer (VLBI) measurements of the QSOs, with the European Network at 1,666 MHz. We have detected two apparently unresolved (≤ 20 marc s) sources with about the same separation as the optical objects and with flux densities of 44 and 32 mJy. These sources have the same flux density ratio as the small-diameter components detected in lower resolution 5-GHz maps but we see no evidence for the extended features in these maps. Our high resolution results are consistent with the gravitational lens hypothesis.

Our observations of 0957 + 561 were made at 1,666 MHz using the Max-Planck-Institut für Radioastronomie (MPIfR) 100-m telescope at Effelsberg, FRG, the Netherlands Foundation for Radio Astronomy 25-m telescope at Dwingeloo, the Netherlands, and the 76-m Mark IA telescope at Jodrell Bank, UK. The data were recorded with a 2-MHz bandwidth using the Mark II VLBI system⁵ and processed with the MPIfR VLBI processor in Bonn. A hydrogen maser frequency standard was used at Effelsberg and rubidium standards were used at the other sites. A total of 5 h of observations was obtained during the period 2–4 June 1979. Table 1 summarises the observation periods. Observations of the relatively strong sources BL Lac and OQ208 showed that the stability of the ionosphere and the local oscillators was sufficient to allow coherent integrations of up to 20 min with only $\sim 5\%$ loss of amplitude.

The longest baseline, Effelsberg–Jodrell, gives lobe spacings of ~ 55 marc s, almost independent of hour angle during these observations. It is also the most sensitive, with r.m.s. noise of ~ 6 mJy in a single 20-min coherent integration. With this integration time, the observations of 0957 + 561 show two well-resolved peaks in the fringe-frequency spectrum. Their separation varies with interferometer hour angle in a way consistent with the separation of the two optical QSOs, A and B. The fringe rate spectrum obtained with a 20-min integration centred at UT 1750, 2 June, is shown in Fig. 1. There is no significant variation in the flux density of A or B with hour angle on 4 June, the individual flux densities being 38 ± 5 mJy and 30 ± 5 mJy, respectively. Note that these errors are a quadratic sum of random noise and an estimated systematic calibration error of 12%. On the Effelsberg–Dwingeloo baseline we have approximately three times less resolution and observations are $\sim 30\%$ less sensitive. Again, there seems to be no variation in the component flux densities with hour angle, the mean values of A and B being 44 ± 6 mJy and 30 ± 6 mJy. From all the 4 June observations we conclude that both components are unresolved. However, for the single half-hour observation on 2 June the flux densities of A and B measured on the Effelsberg–Jodrell baseline are 62 ± 9 mJy and 43 ± 9 mJy. This might be taken as evidence for resolution, variability or even circular polarisation of the components, but we consider that the apparently greater flux densities measured on 2 June can be ascribed to calibration uncertainties. Our best estimates of the flux densities of A and B are therefore the weighted means of these various values; thus, $A = 44 \pm 4$ mJy and $B = 32 \pm 4$ mJy. Some support for the view that the components are unresolved in all the observations comes from the fact that the ratios of flux densities A/B are never significantly different from each other, the mean ratio being 1.38 ± 0.08 . The sensitivity of the Dwingeloo–Jodrell baseline is one-third that of Effelsberg–Jodrell, and although we detect the source, we are unable to deduce anything further about its structure.

There is no evidence of any other radio component from our VLBI observations; from the Effelsberg–Jodrell baseline we put an upper limit of 20 mJy on any compact structure associated with the NE and W radio components reported by Pooley *et al.*³.

It is not possible to obtain useful absolute positions for components A and B from the individual fringe rates. However,

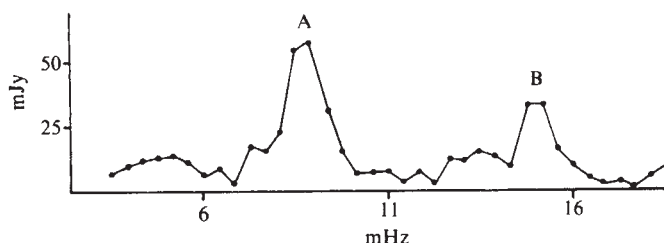


Fig. 1 Residual fringe rate spectrum of 0957 + 561 A, B after 20 min integration. Effelsberg–Jodrell baseline, 17.50 UT, 2 June.

Table 1 List of observation periods

Date	UT	Polarisation
2 June	17.30–18.00	Right hand circular
4 June	08.40–13.00	Left hand circular

we can determine a very precise relative position from the relative phases by using the fringe rate differences to determine the unknown number of integral turns between the phases of A and B. The relative position and standard deviation, in the sense A minus B, from a least squares fit to the relative phases on both baselines, is $\Delta\alpha(1950.0) = -0.1453 \pm 0.0003$ s, and $\Delta\delta(1950.0) = +6.047 \pm 0.002$ arc s. The separation is 6.175 arc s. These values agree well with the 5-GHz results of Pooley *et al.*— $\Delta\alpha = -0.16$ s, $\Delta\delta = 6.1$ arc s (1950.0), separation 6.25 arc s—but are somewhat greater than the optical separation (5.7 arc s) given by Walsh *et al.* Further optical observations are needed to determine whether this difference is real.

In comparing our VLBI results with previous observations, we note first that the 5-GHz flux densities of Pooley *et al.* for A and B (48 and 37 mJy, respectively, flux ratio, A/B, = 1.30) are somewhat higher than those of Roberts *et al.* (36 and 30 mJy, respectively, flux ratio, A/B, = 1.20). These differences may be within the range of observational error. Our VLBI flux densities at 1,666 MHz (44 and 32 mJy, respectively, flux ratio, A/B, = 1.38) are very similar to the 5-GHz values. At first sight the apparent similarity of the spectral indices is further evidence for the hypothesis that we are seeing two images of the same object via a gravitational lens. However, this apparent similarity must be treated with caution for at least two reasons. (1) The 5-GHz observations refer to angular scales of the order of 1 arc s, whereas the VLBI observations refer only to compact components two orders of magnitude smaller, and we do not know what fraction of the 5-GHz flux is contained in these compact components; (2) if a gravitational lens is operating, and if, as suggested by Pooley *et al.*, the source is radio variable, then, taking into account the relative delay between the two paths, we do not necessarily expect the same spectral index for the two images.

The most important feature of our VLBI data is that we find two unresolved radio components with comparable flux density at 1,666 MHz close to the optical QSOs. As concluded by Pooley *et al.*, this is consistent with the gravitational lens hypothesis but because of the much tighter limits on angular size it is a somewhat more stringent test.

If the radio components are, in fact, images of a single object formed by a lens, our failure to resolve them allows an estimate of an upper limit to the size of the object. Adopting the notation and formulae quoted by Pooley *et al.*, the maximum linear magnifications of the two images (transverse to the direction of the line joining them) are α_1/β and α_2/β , respectively. The values of α_1 , α_2 and β can be determined from the flux ratio, which is equal to α_1^2/α_2^2 . Unfortunately, the frequency-independent flux ratio inherent in the geometrical optics of the lens is uncertain because of variability, which, as discussed by Pooley *et al.*, seems to be necessary on lens theory. The closer the flux ratio is to unity, the greater the magnification; thus, a reasonable estimate of the upper limit to the intrinsic size of the radio object may be obtained by adopting the largest value of the flux ratio yet proposed. This is the value derived from the optical spectrum by Carswell and D.W. (in preparation), who suggest that after correction for dust close to the QSO, the flux ratio is 1.5 (in the sense B/A). This then yields $\alpha_1 = 3.40$ arc s, $\alpha_2 = 2.78$ arc s, $\beta = 0.62$ arc s and maximum linear magnifications of 5.5 and 4.5 for B and A, respectively. This implies an upper limit of ~ 4 marc s for the equivalent gaussian diameter of the radio object, and an upper limit to the flux density of ~ 15 mJy if the lens were absent. Note that the formulae from which this result is obtained are based on the assumption of a point deflecting mass.

For the more realistic case of the distributed mass of a galaxy, the precise formulae will depend on the mass distribution, but as long as the flux ratio is not greatly different from unity, the above estimates are probably reasonable.

An upper limit of 20 or even 4 marc s to the diameter of a radio component is quite consistent with that expected for a weak synchrotron source in the nucleus of a QSO, so our size measurements do not provide a definitive test of the lens hypothesis. However, VLBI observations with at least an order of magnitude greater resolution are feasible, and it might then be possible to determine the structures of the two components and so provide a much stronger test of the lens hypothesis.

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Galactic radiation belts

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Several decades of observations have failed to resolve the problem of the interpretation of extended extragalactic radio sources^{1,2}. Most current models invoke the ejection of pairs of plasmoids or relativistic electron beams from the parent elliptical galaxy by various mechanisms^{3–5}. As these theories generally predict the relative orientations of the rotation, magnetic and radio source axes, the measurement of position angles provides one test of the proposed models^{6,7}. However, position angle studies^{8–11} have produced ambiguous and sample-dependent results. While recent analyses^{6,7} indicate a preference for radio source alignment along the inferred rotation axes of the optical counterparts, other investigations found random distributions of position angle or bimodal distributions with peaks separated by $\sim 90^\circ$ (refs 8, 10). One alternative interpretation of extragalactic radio sources that has received little previous consideration, and is considered herein, concerns the possibility that the emission arises from belts of trapped electrons encircling the parent galaxy in the same manner as the Van Allen belts encircle the Earth. This idea seems to have been dismissed primarily because of the absence of ring-shaped or toroidal emission patterns in the radio observations¹². The morphology of synchrotron emission patterns from these hypothetical structures, however, has never been investigated in detail.

An integral describing the two-dimensional brightness distribution on the plane of the sky from synchrotron-emitting relativistic electrons trapped in a dipolar magnetic-field shell was formulated by Ortwein *et al.*¹³ for analysis of the jovian radio emission. These authors derived their results for an arbitrary dipole-axis orientation angle θ_0 with respect to the line of sight and for various degrees of anisotropy of the trapped-electron distribution. The latter was parameterised by the loss-cone angle α_L , which is the smallest populated equatorial pitch angle in the otherwise isotropic particle distribution, and the power law energy spectrum index γ .

While the calculated emission patterns were insensitive to the value of γ , they were strongly dependent on both the aspect

angle and the loss-cone angle. The emitting shell of electrons assumed one of six different morphologies for various combinations of θ_0 and α_L : (1) the solid ellipsoid, sometimes showing complex internal structure, (2) the annulus, (3) the limb-brightened, elongated annulus, which approached in appearance (4) the limb-brightened dumbbell shape, with source axis perpendicular to the dipole axis, (5) the separated double source, with source axis parallel to the dipole axis, and finally (6) the invisible source. Figure 1 shows some of these forms.

For the present investigation, the numerical integrations described by Ortwein *et al.*¹³ were performed for a large number

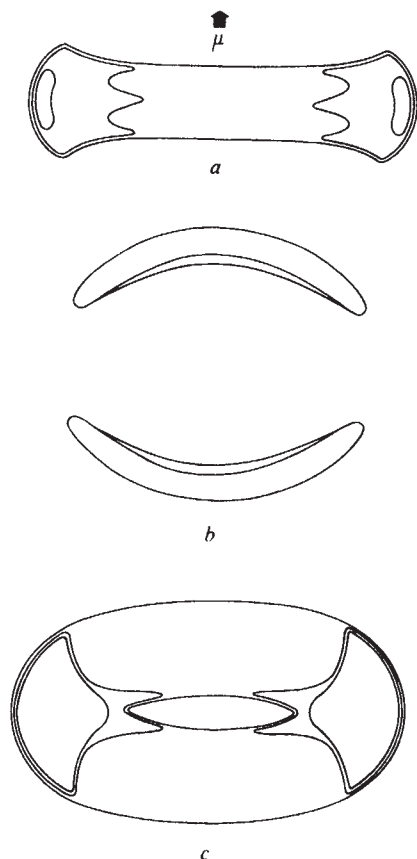


Fig. 1 Examples of the brightness distributions produced by calculations of synchrotron emission from a dipolar shell of trapped electrons using the method described by Ortwein *et al.*¹³. A dumbbell distribution (a) with source axis perpendicular to the dipole moment μ is produced when the aspect angle θ_0 is 90° (line of sight perpendicular to the dipole axis) and the loss cone angle is $\alpha_L = \sin^{-1} 0.8$. A double source with source axis parallel to the dipole axis (b) is obtained when $\theta_0 = 35^\circ$ and $\sin \alpha_L = 0.8$. The limb-brightened, flattened annulus or torus (c) results from the parameters $\theta_0 = 60^\circ$ and $\sin \alpha_L = 0.6$. The contour interval is variable. Numerical results can be found in the work of Ortwein *et al.*¹³.

of aspect angles θ_0 between 0 and 90° and loss cone angles α_L between 0 and 90° . Five-degree intervals were taken in aspect angle θ_0 and $\sin \alpha_L$ was incremented by 0.05. The electron spectral index γ was set equal to the nominal value of 1.5, which is the galactic cosmic-ray index¹⁴. Two-dimensional plots of the intensity were generated for each case. These were then categorised according to morphological class as defined by the six types described above.

Figure 2 shows how the morphology of the brightness pattern varies as a function of aspect angle and electron pitch angle anisotropy. In nature, the dipole axes will be isotropically distributed. However, all loss-cone angles may not occur. In particular, the results given in Fig. 2 indicate that if large loss cones ($\sin \alpha_L > 0.8$) are prevalent, the observed distribution of

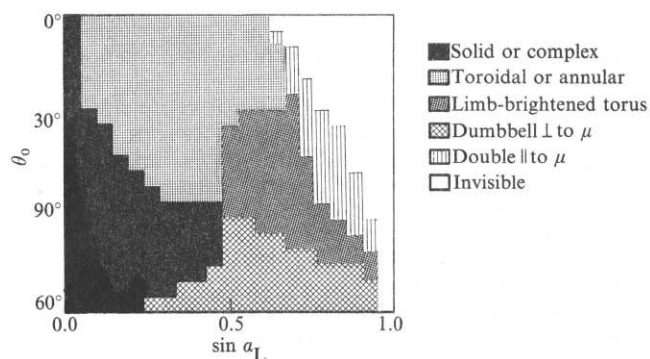


Fig. 2 Distribution of source morphologies for all values of aspect angles θ_0 and loss-cone angle α_L .

source morphologies will be practically equivalent to what has been found in some of the data analyses. These thin disk-like populations of gyrating electrons produce emission patterns that are virtually undetectable or double with the radio source axis either parallel or perpendicular to the dipole axis of the parent galaxy. (The limb-brightened annuli are likely to appear as doubles connected by 'bridges' of emission.)

Of course, actual galactic radiation belts would be composed of a distribution of non-uniformly emitting shells, thus altering the emission patterns somewhat. For example, it would not be surprising to find (under circumstances of non-uniform sources, sinks and field geometry) cases of multiple belts which appear as nearly co-linear paired sources. Also, galaxies with minimum-*B*

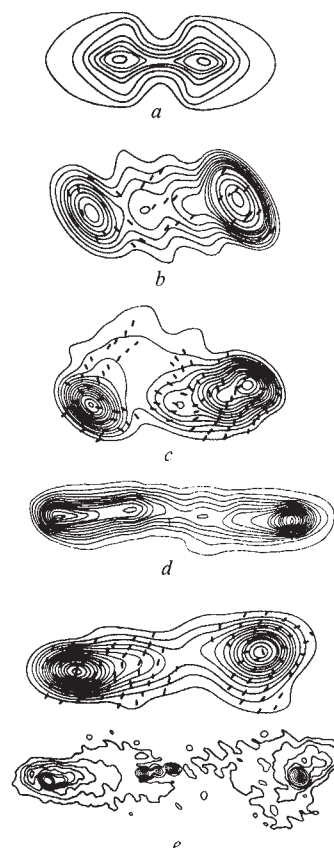


Fig. 3 A selection of observed radio-source brightness distributions at various resolutions: a, Jupiter¹⁷ at 10.4-cm wavelength; b, 3C234 (ref. 16) at 1.4 GHz (21 cm); c, 3C382 (ref. 16) at 1.4 GHz; d, 3C452 (ref. 1) at 1.4 GHz; e, 3C219 (ref. 16) at 1.4 GHz (top) and at 2.7 GHz (bottom). The dashes show the direction of the magnetic field in the sources as inferred from polarisation measurements.

surfaces that are warped by non-dipolar field components¹⁵ may show an anomalous displacement between the rotation and magnetic axes, as well as asymmetric trapping regions. Moreover, a galactic wind²⁴ or local intergalactic material can further distort the configuration of trapped electrons. Figure 3, which compares the brightness distributions and inferred magnetic field geometries of several extragalactic radio sources¹⁶ with the jovian radiation-belt source¹⁷, suggests an intriguing similarity.

The related questions of why galactic radiation belts should exist at all and why they would be populated by electrons with such a highly anisotropic pitch angle distribution lead to several speculations. Parker¹⁸ demonstrated that the magnetic field of our own galaxy could be attributed to dynamo activity. Dipolar components are preferentially excited in dynamos and dominate the field at large distances from the dynamo centre¹⁹. Also galaxies contain sources of energetic particles, the cosmic rays. Various scattering mechanisms and propagation effects^{20,21} can cause particles originating in the galaxy to become trapped in a surrounding dipolar field which is effectively a highly organised cosmic-ray halo. In fact, the cosmic ray spectrum observed in Earth's vicinity could produce the observed radio source spectra²². Finally, Schulz²³ pointed out that both synchrotron losses and radial diffusion processes in radiation belt configurations naturally lead to pitch angle distributions that are strongly peaked at an equatorial pitch angle of 90°.

Many peripheral points of interest can be found in analogies with other radiation belts in nature^{23,25}. The perpendicular sources may show evidence of the gradient-curvature drift of the trapped particles around the parent galaxy. This azimuthal drift would cause opposite Doppler shifts of the synchrotron radiation from the two extremities of the source. Magnetic storms, analogous to those which accelerate particles in Earth's environment²⁶, may have their counterparts in galactic magnetospheres. The variation of the radio sources with time will be determined by the competition between particle sources and synchrotron losses, and also by changes in magnetic field geometry. Some 'tailed' radio sources have already been compared with the Earth's magnetosphere^{27,28}.

These speculations notwithstanding, the calculations described above suggest that electrons trapped in a dipolar magnetic field can reproduce some of the observed distributions of emission from extended extragalactic radio sources, including the absence of toroidal configurations, if the electron pitch angle distributions are sufficiently anisotropic.

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An empirical determination of the heating of the Earth by the carbon dioxide greenhouse effect

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Numerous theoretical calculations^{1,2} have been made of the effect of an increased carbon dioxide concentration in the atmosphere on the surface temperature of the Earth. Estimates of the increase in the surface temperature of the Earth caused by a doubling of the carbon dioxide content of the atmosphere generally range from ~0.7 to 2.9 °C. For a 10% increase in carbon dioxide, the corresponding temperature increases range from 0.096 to 0.40 °C. All these results are calculated from theoretical models which range in complexity from one-dimensional planetary radiation budget studies to three-dimensional general circulation models. Here a new approach to this problem is described which allows an empirical determination of the heating of the surface of the Earth by the carbon dioxide greenhouse effect to be calculated. This technique indicates that the heating of the Earth over the period 1880–1970 was 0.40 °C or less.

Consider that climatic fluctuations have three components: (1) natural climatic changes caused by external forcing such as variations in solar luminosity; (2) natural climatic fluctuations caused by year-to-year stochastic variations in sea-surface temperature, snow and ice cover, cloud cover, and so forth; and (3) man-induced climatic variations caused predominantly by increased carbon dioxide in the atmosphere. Here most of the observed trends in the Earth surface temperature over the last century are hypothesised to have been caused by external forcing, namely by changes in solar luminosity. Once we know the history of this forcing function, the forcing of climate caused by the carbon dioxide greenhouse effect can be determined. If changes in volcanic activity contribute significantly to long-term trends in climate, then the estimate for the carbon dioxide warming will need to be altered. The results of an earlier study³ are now reviewed so that we can reconstruct the solar luminosity variations over the last century.

Sunspots are dark regions on the Sun which vary in number with an 11-year cycle. Nearly all sunspots have a dark central region (umbra) and a less dark surrounding region (penumbra). The areas of these regions have been measured by the Royal Greenwich Observatory since 1874⁴. The ratio of the areas of the umbrae to that of the penumbrae may be used to characterise sunspot structure. The umbral/penumbral ratio varies not only from sunspot to sunspot but also has marked year-to-year variations and shows long-term secular changes (Fig. 1).

The umbral/penumbral ratio may be a measure of the amount of convective energy transport in the photosphere of the Sun^{3,5-7}. Facular activity is more intense when the umbral/penumbral ratio is high⁵, indicating that the umbral/penumbral ratio is directly proportional to the solar convective flux. Deszo⁸ indicates that the manner in which sunspots decay in time also implies that the umbral/penumbral ratio is a measure of solar convective flux. For periods of more active convective motions, the penumbrae tend to be destroyed more readily than the umbrae hence increasing the umbral/penumbral ratio.

If the umbral/penumbral ratio is a measure of convective flux in the Sun, it must also be a measure of radiant flux from the Sun. During periods of more intense convective flux, warmer gases from inside the Sun will rise to the surface and the Sun will be brighter. An earlier study³, using the mixing length theory of convection and available sunspot models, showed that the observed changes in the umbral/penumbral ratio were

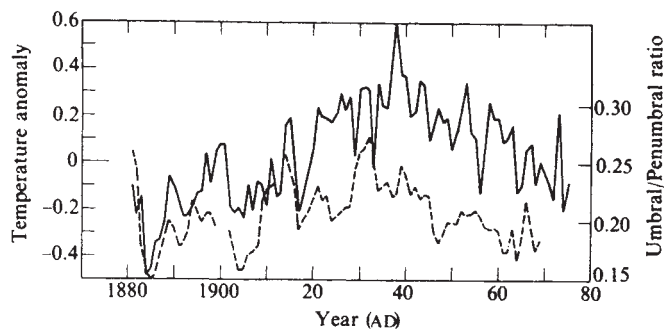


Fig. 1 The solid curve shows the Northern Hemisphere temperature anomalies from Budyko (1881–1957), Reitan (1958–1968) and Angell and Korshover (1969–1975). The dashed curve illustrates the umbral/penumbral ratio from the Royal Greenwich Observatory measurements 1881–1969. Note the general similarity, particularly for the earlier years.

equivalent to a change on the solar constant of about 0.4% over the last century.

A consequence of the above is that the climate of the Earth will vary in a parallel manner with variations of the umbral/penumbral ratio. The higher the umbral/penumbral ratio, the greater will be the solar constant and the warmer the Earth. Figure 1 compares the temperature anomalies of the Northern Hemisphere^{9–11} and the umbral/penumbral ratio. The cross-correlation of these two time series is 0.53 ± 0.13 which is significant at better than the 1% level. The uncertainty of this correlation has been increased¹² to correct for the autocorrelation in the two time series. The umbral/penumbral time series has been corrected for the years 1900, 1901 and 1913 as suggested earlier³, although using years with few sunspots causes the correlation to be less than it would be otherwise. Using only those years for which the penumbra covers more than 100 millionths of the solar disk gives a cross-correlation of 0.57. The probability of such a high cross-correlation occurring by chance is < 1 in 10^2 . Furthermore, when both series are detrended by fitting parabolas to each and subtracting them from the observed time series, the cross-correlation of the residuals equals 0.46. It is the common year-to-year variations in the two time series that gives rise to their correlation rather than their common long-term trends.

Although the umbral/penumbral ratio successfully accounts for ~28% of the total variance in the observed temperature records, the crucial test of this mechanism for climatic change is that solar constant variations be proportional to the umbral/penumbral ratio. The predictive capability of the model as it stands supports its validity. In particular, it has successfully predicted the following: (1) the umbral/penumbral ratio should be small during the Maunder Minimum of the late 1600s (ref. 13). A preliminary qualitative check of available sunspot drawings agrees with this prediction³. (2) Because of the need for energy balance in the solar convective zone, the solar rotation rate should have increased in recent years: Measurements by Livingston and Duvall¹⁴ confirm this prediction. (3) Correspondingly the solar rotation rate before 1930 when the Earth was warming should have decreased to a minimum about this time: measurements by Eddy¹⁵ apparently confirm this prediction. (4) Because of a 20-yr cycle in the umbral/penumbral ratio³, there should be a 20–22-yr cycle in certain meteorological parameters. The best documented such variation concerns the 22-yr cycle of droughts in the western US¹⁶. The umbral/penumbral ratio has peaked 0–4 yr before each drought over the past century³ and strongly suggests a physical connection. (5) The thermal time constant of the Earth–ocean–atmosphere system should be ~4–5 yr based on calculations using a simple energy balance climate model. Others^{17–18} have estimated this time constant to be ~6.5–6.9 yr. Further information on this hypothesis for climatic change is given elsewhere³.

The proposed hypothesis should also allow a successful prediction of the warming of the Earth by the carbon dioxide greenhouse effect. Only three basic assumptions are needed (1)

the umbral/penumbral ratio and surface temperature of the Earth are correlated and proportional to one another. Although for physical reasons the umbral/penumbral ratio is believed to provide a measure of the solar constant whose variations in turn cause climatic change, another physical mechanism could be invoked without upsetting the above assumption. The record for the surface temperature of the Northern Hemisphere is taken to be representative of the whole Earth¹⁹ although doubts have been expressed about the parallelism of the temperature records of the two hemispheres²⁰. (2) The carbon dioxide content of the atmosphere has been increasing exponentially since 1880 (refs 21, 22) and consequently the temperature of the Earth has been increasing approximately exponentially with time¹. (3) Earth surface temperatures have long-term secular variations over the past 100 yr forced predominantly by variations in solar luminosity and carbon dioxide increases. Long-term trends in atmospheric transmission attributable to anthropogenic or volcanic aerosols reported by Budyko⁹ as a possible cause of climatic change have not been supported by many subsequent studies^{29–33}. Volcanic activity or natural variations may contribute to the trend in temperature but for the first estimate of the carbon dioxide warming these effects are neglected.

The trends in the observed temperature record^{9–11} (Fig. 1) with the above assumptions consist of a natural variation caused largely by solar variations and anthropogenic portion caused by increased carbon dioxide. The anthropogenic portion is analytically represented in two models by a linear and exponential increase of temperature with time. Subtracting various hypothetical anthropogenic temperature increases from the observed temperature records allows natural temperature variations to be deduced. The highest cross-correlation of a calculated natural temperature variation versus the umbral/penumbral ratio is 0.63 and occurs for an assumed linear anthropogenic temperature increase of 0.40 °C over the period 1880–1970 (Fig. 2) or assumed exponential increase of 0.3 °C to 0.4 °C. The deduced increase in temperature caused by anthropogenic carbon dioxide should be interpreted as an upper limit to the actual warming because the presumed cooling in the late 1880s caused by volcanic eruptions²³ will tend to be interpreted as a natural climatic variation. Hence, the warming after the volcanic eruptions will give a higher value for the calculated carbon dioxide warming than it should. A more detailed climate model should help resolve the magnitude of this overestimation. The location of temperature measurements near urban areas may also give the appearance of a long-term warming of the Earth because of the increasing effects of urban heat islands¹⁹. The observed and expected natural temperature variations in the absence of mankind are shown in Fig. 3.

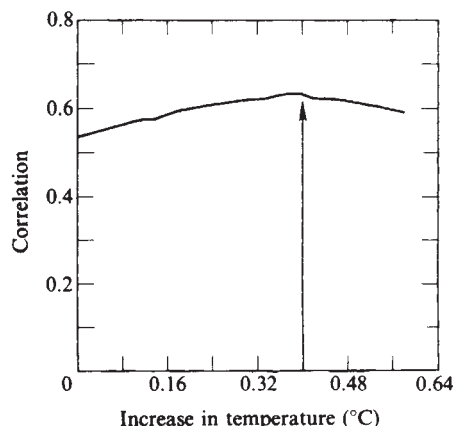


Fig. 2 The correlation of the natural temperature anomalies compared with the umbral/penumbral ratio for various assumed linear temperature increases caused by the carbon dioxide greenhouse effect during 1880–1970. The best correlation occurs for a linear temperature rise of 0.4 °C. A similar plot for a more realistic exponential temperature rise gives a best correlation for 0.3–0.4 °C.

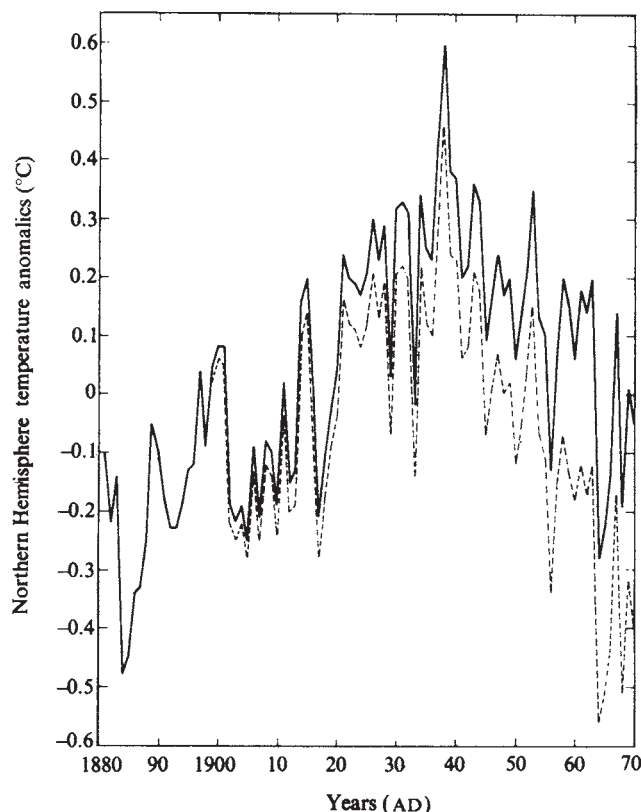


Fig. 3 The solid curve gives the observed Northern Hemisphere temperature anomalies and the dashed curve gives the natural unperturbed Northern Hemisphere temperature anomalies. The increasing exponential separation of the two curves is caused by the increased (anthropogenically produced) carbon dioxide.

Although the 0.30–0.40 °C warming due to the carbon dioxide greenhouse effect is the present best estimate of this quantity based on techniques used here, there is considerable uncertainty in the statistical significance of this result. The cross-correlation between the calculated externally driven natural temperature variation and the umbral/penumbral ratio is 0.63 ± 0.13 . The cross-correlation between the observed temperature variation and ratio is 0.53 ± 0.13 . As only 90 yr of data are used in calculating these correlations, the significance of the difference between the correlations is only 16% (ref. 24). Although this technique does not prove that the Earth is warmer because of the presence of added carbon dioxide in the atmosphere, it supports this hypothesis.

Estimates of the carbon dioxide content in the atmosphere in 1880 range from 280 (ref. 21) to 293 p.p.m. (refs 23, 25). If during 1970 the concentration was 320 p.p.m. (ref. 26) the total increase in carbon dioxide from 1880 to 1970 was 9–14% of the original amount. The 0.40 °C temperature increase therefore implies that doubling the amount of carbon dioxide would result in warming in the range 2.0–3.1 °C. Manabe and Wetherald^{27,28} estimate a 2.4 and 2.9 °C temperature increase respectively for the same conditions. The Manabe and Wetherald models imply that for a 9–14% increase in carbon dioxide, the Earth's surface temperature increases by 0.30–0.55 °C which bracket the present 0.3–0.4 °C empirical prediction. The present results further predict that a continued exponential increase in carbon dioxide content of the atmosphere, will warm the Earth by an additional ~0.3–0.4 °C by the year 2000.

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Evidence for quasi-periodic July drought in the Hudson Valley, New York

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July Palmer Drought Severity Indices¹ (PDSI) have been reconstructed² for the Hudson Valley region of New York State from the annual ring-width variations of local old trees. Using additional, subsequently developed tree-ring chronologies, we have developed a new July drought reconstruction that extends back to 1694. Variance spectra of the new PDSI series reveal statistically significant quasi-periodicities of 11.4 and 26 yr.

The July PDSI reconstruction was developed using six tree-ring chronologies from sites in the Hudson Valley. Each chronology is represented by only one tree species and is a mean-value function of 30–40 ring-width series from individual trees on a site. Five tree species are represented in the six chronologies: eastern hemlock (*Tsuga canadensis* Carr.), pitch pine (*Pinus rigida* Mill.), eastern white pine (*P. strobus* L.), chestnut oak (*Quercus prinus* L.) and white oak (*Q. alba* L.). Using several species and sites practically eliminates the possibility that insect infestations or other non-climatic disturbances have created the fluctuations found in the drought reconstruction. The reconstruction was developed using a combination of principal components analysis and multiple regression to compute prediction equations for estimating past climate^{3,4}.

Figure 1 shows the tree-ring PDSI estimates (a) plotted against the actual Hudson Valley regional July PDSIs (b). The latter series was used as the dependent variable to develop the multiple regression calibration equation for reconstructing drought. The estimates reduce 54% of the variance in Fig. 1b adjusted for degrees of freedom⁵. This is statistically significant well below the 1% level. The July PDSIs in Fig. 1c were computed from monthly temperature and precipitation data of the Mohonk Lake, New York cooperative meteorological substation which is near some of the tree-ring sites. The 1896–1930 interval is independent of the calibration period and, thus, can be used to test and verify the accuracy of the regression estimates. Three tests of verification were used: the sign test, the product means test³ and the degree of cross-correlation. The sign test (30 agreements, 5 disagreements) and the correlation coefficient ($r = 0.60$; d.f. = 33) pass the 1% significance level. The product means test ($t = 2.21$; d.f. = 33) passes the 2.5% significance level.

To test the frequency-domain fidelity of the reconstruction, we computed spectral and cross-spectral estimates⁶ of the

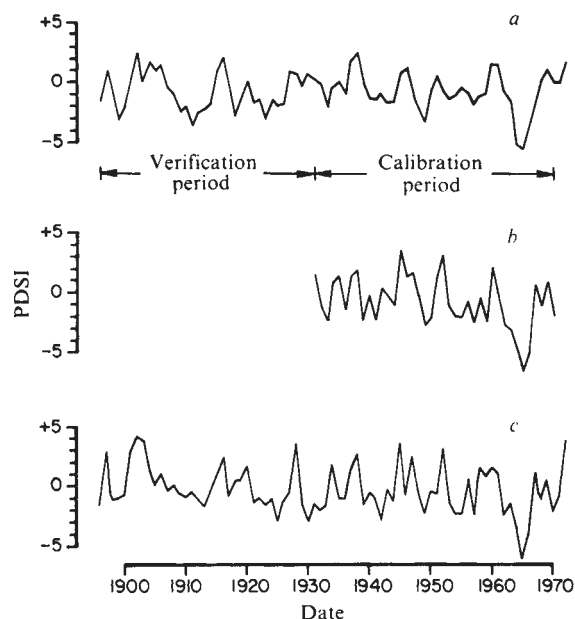


Fig. 1 Tree-ring reconstructed July PDSI (a), Hudson Valley Division July PDSI (b) used to calibrate the multiple regression equation for estimating PDSI from tree growth, and Mohonk Lake, New York, July PDSI (c) used to verify the tree-ring PDSI estimates.

Mohonk Lake and reconstructed PDSI series. For this test we used all of the common data (1896–1972) to provide better low-frequency resolution. The two series are no longer independent because the Mohonk Lake data are highly correlated with the divisional data used in the 1931–70 calibration period. But as the 1896–1972 common interval is almost twice the calibration period, we still expect a reasonably rigorous depiction of the frequency-domain fidelity of the PDSI reconstruction.

Figure 2 shows the variance spectra (a) and the squared coherency spectrum (b) of the two series. The similarity of the variance spectra illustrates the good fidelity of the drought signal recorded by tree rings. The loss of variance in the higher frequencies of the reconstruction is partly a function of the unexplained variance of the regression equation used for prediction. Note that both spectra have a broad-based peak centred at a period of 12.6 yr.

The coherency spectrum provides an estimate of the common per cent variance between two series at each frequency. This spectrum indicates that 60–80% of the climatic signal in the bandwidth accounting for periods of 7–30 yr is common to both series. Other frequencies show poor to good coherence. The phase spectrum is not shown because the two series are in phase for almost all frequencies.

Figure 3 shows the complete July PDSI reconstruction and a low-pass filtered version that passes all variance at frequencies of once in 10 yr or less. Judging from the coherency spectrum, the filtered PDSI series shows the most reliable drought information. It clearly shows a strong low-frequency rhythm of wetness and dryness that is suggested in Fig. 2.

Using the 1700–1972 period of the unfiltered series, we computed a high-resolution variance spectrum from the autocovariance function of $N/3$ or 91 lags. The Hamming window was used for smoothing the spectrum. Figure 4a shows two very strong peaks of variance at periods of 11.4 and 26 yr. The general shape of the spectrum is characteristic of a time series containing 'red noise' or persistence which inflates the low-frequency variance. Significance tests of such spectra must be computed either from a null spectrum of an empirically determined low-order Markov process⁷ or after prewhitening to

reduce the original data to residual 'white noise'. We use both approaches with an *a priori* hypothesis test, but compute the confidence levels from somewhat different null models.

The null spectrum of the unwhitened PDSI data was computed from the autocovariance function of 14 lags which produces a very low resolution spectrum. In this case, we want the null spectrum to be arbitrarily smooth without defining the stochastic process it represents. Using a one-tailed χ^2 test, the significance levels are computed as

$$CL = \frac{\Gamma(\omega) \cdot \chi^2}{\nu} \quad (1)$$

where $\Gamma(\omega)$ is the null spectrum estimate for frequency ω and χ^2 is the χ^2 value for a given confidence level (CL) with ν degrees of freedom. In Fig. 4a, the 95 and 97.5% confidence levels are plotted with the high and low resolution spectra. Both the 11.4- and 26-yr peaks exceed the 97.5% level of significance.

In Fig. 4b, the high-resolution spectrum was computed after the PDSI series was prewhitened using the difference equation of the second-order autoregressive (AR) process

$$Y_t = A_t + 0.827 Y_{t-1} - 0.293 Y_{t-2} \quad (2)$$

The order of the process was selected using the Akaike final-prediction-error criterion⁸ which is an unbiased estimator of the order of an AR process⁹. Although the drought series may be more complex than an AR (2) process, the cumulative periodogram of the prewhitened series did not show any systematic departure from a white noise null continuum. For white noise

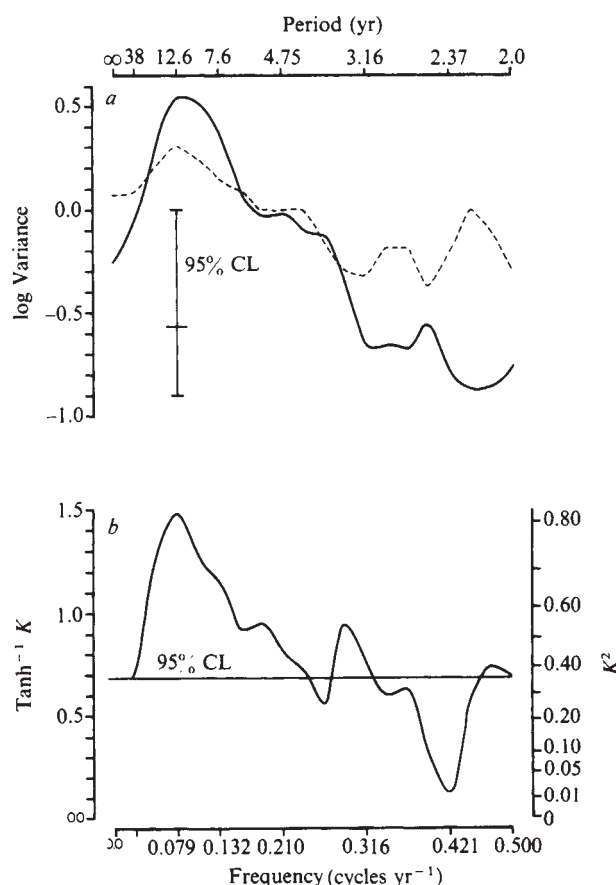


Fig. 2 Variance spectra of Mohonk Lake and tree-ring reconstructed July PDSI and the squared coherency (K^2) spectrum of the two series. Data shown in Fig. 1a and c were used to compute the spectra. The confidence interval of each variance spectrum is the same. $N=77$; d.f. = 10; bandwidth = 0.066. Dashed line indicates Mohonk Lake, New York, July PDSI.

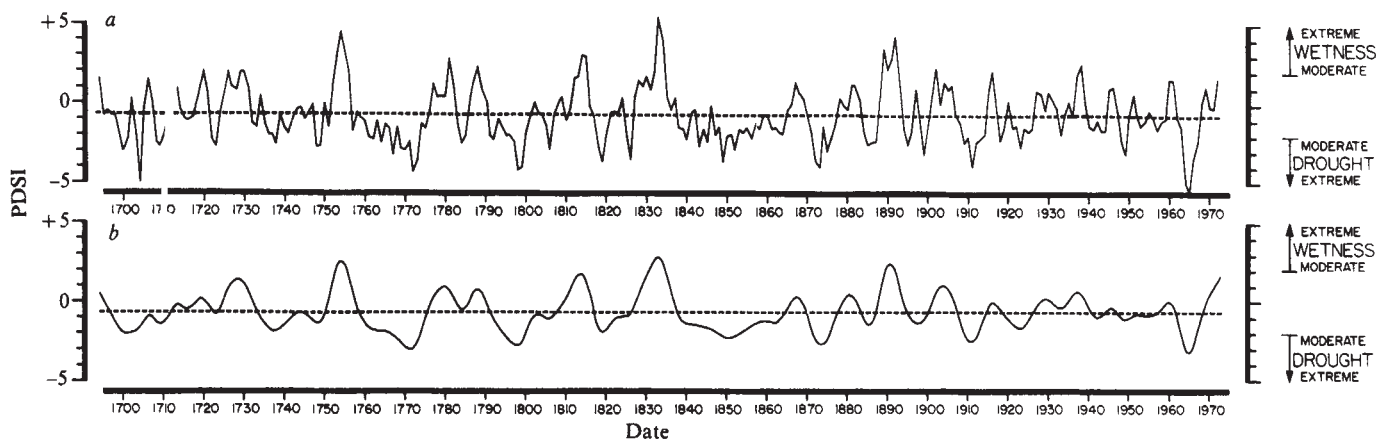


Fig. 3 Tree-ring reconstructed July PDSI from 1694 to 1972. *a*, The unsmoothed estimates. *b*, A low-pass filtered version of the unsmoothed series that emphasises frequencies of once in 10 yr or less.

processes, the expected relative variance of the null spectrum at each frequency is

$$\Gamma(\omega) = 1.0/m \quad (3)$$

where m is the number of spectral estimates. $\Gamma(\omega) = 0.011$ for all ω in our case and the confidence levels are computed using equation (1). Again the 11.4- and 26-yr peaks are significant at the 97.5% confidence level.

We conclude that low-frequency fluctuations of past drought in the Hudson Valley are partly quasi-periodic. These periods

may be identified with the 11-yr sunspot cycle¹⁰ and a 27-yr cycle of soli-lunar tidal influence on zonal westerly flow¹¹. An unsmoothed periodogram of the drought series indicates that ~20% of the variance is accounted for by these harmonics. Although this percentage suggests that these harmonics may have some practical predictive use, the nature and cause of these signals must first be understood. We strongly discourage any idea that drought in the Hudson Valley can be operationally predicted from the present results.

The belief that influences outside the Earth's atmosphere could significantly modulate climate is gaining credibility. A rigorous statistical analysis has convincingly linked the 22-yr midwestern US drought rhythm with the Hale sunspot cycle and its envelope, the Gleissberg cycle¹².

A highly coherent spatial pattern of inferred solar cycle signal (~10.7 yr) has also been detected in surface-air temperature data over North America¹³. It is particularly interesting that the presence of that signal is apparently limited to the region north of 35° N latitude and east of the Rocky Mountains divide. The Hudson Valley is within this region.

We do not yet know if there are consistent relationships between our drought series and such external forcing functions of climate. However, the 11.4- and 27-yr quasi-periodic drought rhythms are sufficiently near the soli-lunar tidal and sunspot periodicities to warrant a statistical inquiry into these possible relationships. Even if these harmonics are eventually regarded as part of an ensemble of internal stochastic mechanisms¹⁴ of the ocean-atmosphere-cryosphere system, they represent an important aspect of climatic variability that is still poorly understood.

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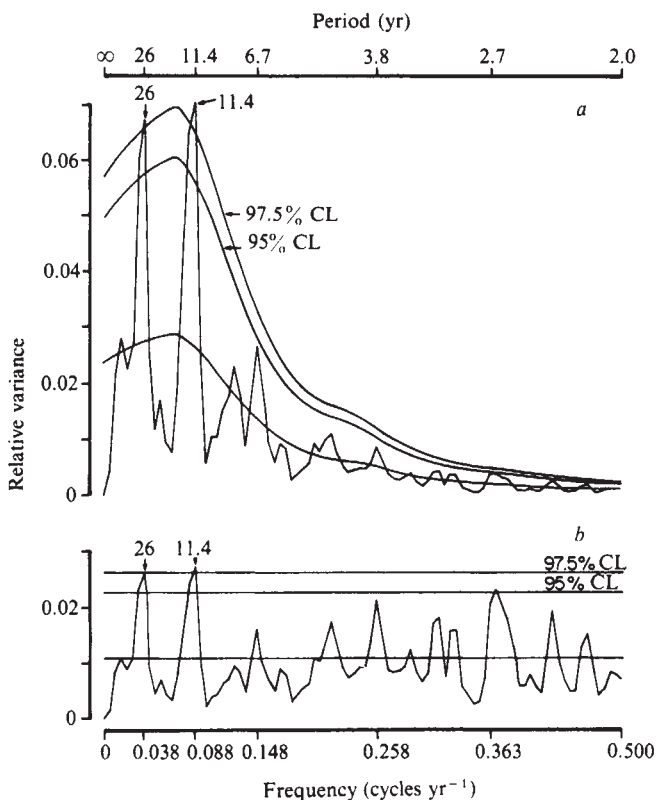


Fig. 4 Variance spectra of the unsmoothed July PDSI reconstruction (*a*) and the same series after prewhitening (*b*) using equation (2). An appropriate null continuum and its 95% and 97.5% confidence levels (CL) are superimposed on each spectrum. The bandwidth = 0.0138 and d.f. = 6 are the same in each case. $N = 273$.

A search for superheavy-element fission-tracks in iron meteorites

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In a series of recent papers Runcorn and coworkers¹⁻³ have hypothesised that the radioactive decay of siderophile superheavy elements (SHE) provided the heat source which produced early melting of the Moon and which drove an ancient lunar core dynamo. If such elements had a half life of $\sim 10^8$ yr then this provides a natural explanation for the decay of the postulated lunar core dynamo and the associated magnetic field on a time scale of this order. It would be surprising if these SHE had accreted into the early Moon and not into the parent bodies of the iron meteorites. Libby *et al.*⁴ have suggested that the iron meteorites did accrete SHE, and have argued that SHE fission has produced the observed peaks in the trace element abundances in iron meteorites at charge number $Z \sim 40, 50$ and 80 . Libby *et al.*⁴ claim that these abundances are well above those which may be expected on the basis of a simple calculation of the solubilities in Fe of these elements. These peak trace element abundances are ~ 10 p.p.m. by weight and assuming an element at the peak corresponds to a fission yield of 5% then the original abundance of SHE in the metal must have been in the region of 2×10^{-4} kg kg⁻¹. Such high concentrations of fissioning elements should produce a number of observable effects, one of which is the production of very large numbers of radiation damage tracks in the borders of silicate grains along the boundary with metal regions. This boundary contribution falls to zero at a distance within the silicate crystal equal to one fission fragment range (~ 5 – 10 μ m). We report here a search for fissioning species in the metal phases of IA iron meteorites and a pallasite. No excesses of tracks in these boundary regions have been found and an upper limit of $\sim 10^{-12}$ kg kg⁻¹ can be placed on the concentration of fissioning superheavy elements in the metal of the Odessa IA iron at the time when fission track retention began.

We have examined the silicate portions of two group IA iron meteorites and of the Brenham pallasite. In the case of the iron meteorites Odessa and Landes, slices were cut and the metal-silicate boundaries examined. A large Brenham olivine inclusion was extracted cleanly from the metallic matrix and mounted with the interface surface uppermost. One slice of Brenham was also studied.

Track densities near to the centre of each grain and along a strip ≈ 10 μ m wide immediately adjacent to the metal contact were measured. In addition track densities on the interface surface of one olivine grain were also measured. The track densities are displayed in Table 1. Also given are the probable sources of the tracks in the central regions of the crystals.

Various criteria for deciding on the principal sources of tracks in any given meteorite crystal have been enumerated by Fleischer *et al.*⁶. In Brenham olivine, orthopyroxene from Landes and feldspar from the Odessa meteorite the tracks are observed to have anisotropic angular distributions and track length distributions dominated by short (< 5 μ m) tracks. These tracks would seem to result from slowed-down heavy cosmic-ray primaries. Track densities encountered in Odessa diopside are considerably higher than those in nearby feldspar crystals. The angular distribution of the tracks is isotropic and the length distribution fairly flat up to track lengths of ~ 12 μ m (ref. 7). These characteristics all indicate a fission origin for these tracks⁶ and previous work⁷ has established that the majority are most probably formed by the spontaneous fission of ²⁴⁴Pu.

These assignments of track origin are important because they determine the density of tracks to be expected in the boundary

regions. For an external source such as cosmic rays the mean track density measured in a 10 - μ m wide strip at the silicate-metal boundary, ρ_B should equal the central track density ρ_C . For an internal source such as fission the track density on the external surface of a silicate grain should be $\sim \rho_C/2$ rising to ρ_C at one fission fragment range (~ 10 μ m) within the crystal. Over a ~ 10 - μ m boundary strip the mean track density which will be measured will be approximately $0.75\rho_C$. This estimate is only a rough indication of the expected track density since the exact value will depend for example on the angle made by the metal-silicate interface to the sample surface and also on the magnitude of the contribution of external track sources, nevertheless, it gives a rough guide for comparison with the experimental results. Columns 4 and 5 of Table 1 show expected and measured track densities in the boundary regions. Figure 1 shows a scanning electron micrograph of a typical metal-silicate boundary.

The results of Table 1 show no evidence for an excess of tracks near to the metal-silicate boundary, except for an abundance of very shallow pits on the interface surface of the Brenham olivine grain which were not visible on internal surfaces. These did not have the appearance of fission tracks under the optical or scanning electron microscopes, being < 1 μ m deep. The most plausible explanation for their appearance is that they are spallation recoil tracks resulting from cosmic ray interaction with heavy elements, such as Ni, in the metal phase.

The lack of excess tracks in Brenham suggests an upper limit on the SHE track density of the same order as the errors in the track density measurement, $\sim 2 \times 10^3$ cm⁻². Landes orthopyroxene and Odessa feldspar and diopside allow rather less restrictive limits to be placed on the SHE track density contribution because of their higher central track densities.

It is difficult to interpret these track densities in terms of limits on the SHE concentrations unless the cooling histories of the meteorites are known. For example, if Brenham was affected by a late heating event then pre-existing fission tracks could be removed. If the time of track retention began sufficiently recently then most of the SHE would have decayed. Runcorn has estimated a half life of $\sim 10^8$ yr for the proposed superheavy elements on the basis of the decay of the lunar palaeofield.

In the case of Odessa diopside, however, it has been shown⁷ that the majority of the tracks are due to fission of ²⁴⁴Pu. The track density ratio $\rho_{Pu}/\rho_U = 106 \pm 9$. The age implied by such a ratio depends on the initial Pu/U ratio. In meteoritic whitlockites Kirsten *et al.*⁸ have found Pu/U ratios at the onset of Xe

Table 1 Measured and expected boundary track densities

Meteorite [Class]	Mineral [no. of crystals examined]	Central track density (cm ⁻²) [probable source]	Expected boundary track density (cm ⁻²)	Measured boundary track density (cm ⁻²)
Brenham [pallasite]	Olivine* [2]	$(1.0 \pm 0.2) \times 10^4$ [cosmic rays]	$(1.0 \pm 0.2) \times 10^4$	Deep pits $\sim 10^4$ Shallow pits $\sim 2 \times 10^5$
Landes [IA iron]	Ortho- pyroxene† [2]	$(1.50 \pm 0.18) \times 10^7$ [cosmic rays]	$(1.50 \pm 0.18) \times 10^7$	$(1.47 \pm 0.29) \times 10^7$
Odessa [IA iron]	Feldspar‡ [1]	$\sim 10^5$ [cosmic rays]	$\sim 10^5$	$\sim 10^5$
Odessa [IA iron]	Diopside§ [3]	$(9.05 \pm 0.27) \times 10^6$ [fission of ²³⁸ U and ²⁴⁴ Pu]	$(6.79 \pm 0.20) \times 10^6$	$(5.47 \pm 0.43) \times 10^6$

* Etchant, boiling WN⁵ solution at pH 8 for 2–4 h.

† Etchant, 60 wt % boiling aqueous NaOH solution for 1 h.

‡ Etchant 40 wt % boiling aqueous NaOH solution for 1 h 40 min.

§ Etchant as for feldspar + 30 min in 60 wt % boiling aqueous NaOH solution. All etching stages carried out under reflux.

|| Track densities measured using Leitz ortholux optical microscope at 630× magnification, except for shallow pits in Brenham olivine which were measured under Cambridge Mk IV SEM at 5,000× magnification. The SEM was also used to check on the integrity of metal-silicate boundaries.

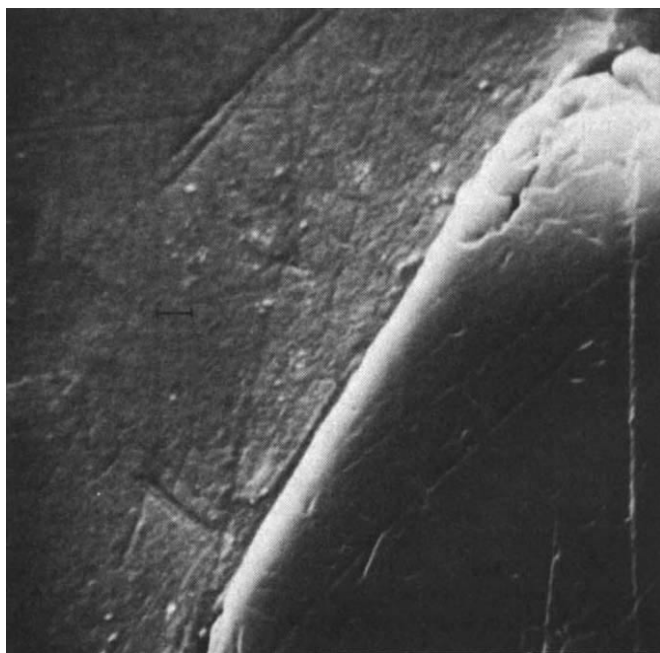


Fig. 1 Scanning electron micrograph showing the boundary between metal (left hand side) and a diopside crystal (right hand side) in a slice from the Odessa iron. The slice was first etched in 40 wt % aqueous NaOH solution for 100 min to develop tracks in feldspars. Tracks in diopside were then revealed after a further 30 min of etching in 60 wt % NaOH solution. The scale bar represents 1 μm . There is no indication of a very high track density at the boundary or of the disintegration which would result from the etching of an intensely radiation damaged boundary region.

retention of up to 0.13. Fractionation trends for Pu and U in diopside are not well known, however, if we take an extreme upper limit for Pu/U of 0.5 at 4.58×10^9 yr ago then the diopside track densities in Odessa still imply track retention as long ago as $\sim 4.2 \times 10^9$ yr. This corresponds to ~ 4 half lives after the formation of the Solar System for the postulated SHE if we assume with Runcorn^{1,2} a half life of $\sim 10^8$ yr. With an initial SHE concentration of $2 \times 10^{-4} \text{ kg kg}^{-1}$ in the metal phase then at this track retention time there would be $\sim 10^{-5} \text{ kg kg}^{-1}$ still remaining. If this quantity has decayed to extinction via fission then the number of fission tracks crossing 1 cm^2 of a metal-silicate boundary would be $\sim 5 \times 10^{13}$. (The number of tracks per cm^2 , ρ , on the silicate surface in contact with the metal will be given approximately by $\rho = 1/2(CN_0R/A)$ where C is the weight fraction of SHE in the metal, R the fission fragment range in g cm^{-2} , N_0 is Avogadro's number and A is the atomic weight of the SHE.) This is an enormous track density probably sufficient to render a $\sim 10\text{-}\mu\text{m}$ layer of silicate totally amorphous⁹. Failure to observe track excesses at the level of $\sim 10^6\text{--}10^7 \text{ cm}^{-2}$ therefore implies that the fissioning superheavy content of the Odessa metal was at least seven orders of magnitude lower than those inferred from trace element data by Libby *et al.*⁴. If instead we take as the SHE abundance that estimated for the lunar core at $\sim 4.2 \times 10^9$ yr ago (see Fig. 1 of ref. 4) that is, $\sim 10^{-7} \text{ kg kg}^{-1}$ then we arrive at an expected boundary track density of $\sim 5 \times 10^{11} \text{ cm}^{-2}$, still five orders of magnitude above a level which would be compatible with the observations reported here.

In the case of Odessa diopside, observation of ^{244}Pu fission tracks implies that no annealing event can be invoked which would remove the SHE fission track excess as this would remove the ^{244}Pu fission tracks. Of course, if the SHE had a half life of $\ll 10^8$ yr then a thermal history could be constructed which would remove the SHE tracks but leave the bulk of the ^{244}Pu tracks intact. Presumably such SHEs could not then be those which would drive a lunar core dynamo for several hundred million years. The SHE abundances could be made compatible

with the observations provided that we accept a somewhat contrived model, namely that the silicates and metal were not placed into intimate contact until $\sim 10^9$ yr ago. Radiometric ages for the silicate inclusions in irons are generally $\sim 4.6 \times 10^9$ yr (ref. 10) and none is yet known to be younger than 3.8×10^9 yr. Such an emplacement event therefore would have to be such that the Rb-Sr, K-Ar ages were not reset.

Taking as a generous estimate for the upper limit to the boundary track excess the actual measured boundary track densities of Table 1, column 5, then the corresponding upper limits to the concentration of fissioning SHEs become $\sim 2 \times 10^{-15} \text{ kg kg}^{-1}$ for the metal phase of Brenham (from the density of deep etch pits) and $\sim 10^{-12} \text{ kg kg}^{-1}$ for metal adjacent to Odessa diopside. These figures apply to the time at which quantitative retention of fission tracks began in the silicates. This is probably not later than $\sim 4.2 \times 10^9$ yr ago in the case of Odessa diopside but this is not known with any certainty for the case of Brenham olivine. Of course, the presence of α -decaying super-heavy elements is not precluded by these data but if α -decay is the predominant mode of decay for the SHE then the similarity between trace element abundance peaks and fission product abundance curves pointed out by Libby *et al.*⁴ must be purely fortuitous.

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Amino acids in the Yamato carbonaceous chondrite from Antarctica

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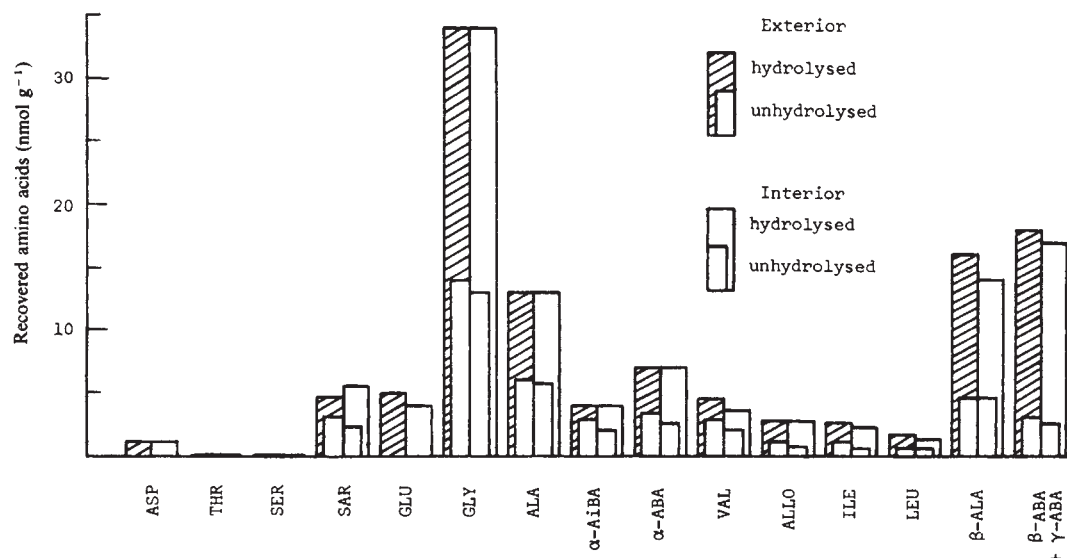
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More than one thousand pieces of meteorites have been discovered recently in Antarctica¹⁻⁴ which were preserved in the Antarctic Ice Sheet since their fall and were brought out on to the surface of the bare ice. They are likely to have the least possible terrestrial contamination, thus providing a new opportunity for the study of primordial organic synthesis in the early solar nebula⁵ and of chemical evolution before the emergence of life^{6,7}. Organic compounds of extraterrestrial origin were first detected in the form of protein and non-protein amino acids with an equal abundance of their D- and L-enantiomers in the Murchison carbonaceous chondrite^{8,9}. This ratio of enantiomers has been used as a criterion for the meteoritic origin of the amino acids detected subsequently in other meteorites¹⁰⁻¹³. We present here detailed evidence for the presence of amino acids of extraterrestrial origin in our Antarctic carbonaceous chondrite¹⁴.

Fig. 1 Amino acids recovered from the Yamato meteorite 74662. The quantities were estimated by the amino acid analyser. The amount of β -ABA + γ -ADA consists mostly of γ -ABA by the gas chromatographic study.



A ~5-g fragment of the original 150-g meteorite was separated into exterior, middle, and interior portions. These portions were pulverised and nearly 1 g of the exterior and interior was individually extracted with water at 105 °C for 24 h. The water extracts were centrifuged and the supernatants were recovered. The residues were rinsed twice with water and combined with the corresponding supernatants. Half of each resulting solution was further halved into two aliquots. One of the aliquots was reduced to an appropriate volume and analysed by a Durrum 500 amino acid analyser. The other aliquot was dried under an IR lamp and derivatised, first, with isopropyl alcohol, 1.5 M HCl, and, then, TFAA-CH₂Cl₂ (1:2 by volume). The N-TFA-isopropyl-derivatives of amino acids were analysed by a gas chromatograph (GC) equipped with a Chirasil Val glass capillary column (25 m) using a nitrogen detector. The other half of the resultant solution was evaporated and was hydrolysed with 6 M HCl at 105 °C for 24 h. The hydrolysed solution was divided into two equal portions which were dried completely under the IR lamp. One of the dried samples was redissolved with an appropriate volume of water and analysed by the amino acid analyser. The other dried sample was subjected to the derivatisation for the enantiomer study by GC. All analytical preparations were conducted in a class 100 clean room.

Fifteen amino acids were detected in the Yamato meteorite: of these, nine are protein and six are non-protein amino acids. The individual amino acids have abundances between 34 and <1 nmol per g meteorite. Figure 1 compares the exterior versus interior and the unhydrolysed versus hydrolysed portions. The abundances in the exterior and interior are similar. The yields of amino acids increase two to three times after the acid hydrolysis.

The gas chromatogram obtained with the nitrogen detector shows a complex mixture. However, the D- and L-enantiomers of alanine, aspartic acid, and glutamic acid have nearly equal abundance. If other overlapping peaks around the baseline level are subtracted this fact might become even more evident (Fig. 2). The nearly equal abundance was observed in all the unhydrolysed and hydrolysed portions of the exterior and interior of the meteorite.

The amino acid assemblage, their concentrations and the enantiomeric ratios found are indicative of amino acids of abiotic origin and, therefore, extraterrestrial in nature. It may be argued that the observed DL ratios were produced during the preservation of the meteorite after contamination with terrestrial biological material at the time of fall. Unlike relatively recent falls of the Murchison (1969), Murray (1950), Orgueil (1864) and Mighei (1889) that exclude a pronounced racemisation during the terrestrial age, Antarctic meteorites might have enough time for an extensive racemisation. The age of the present bare ice surface of the Yamato Mountains may be

240,000 yr (ref. 2). However, as these meteorites fell on, and were subsequently preserved in, the Antarctic ice the terrestrial contamination would be insignificant and the racemisation process must have been exceedingly slow. The presence of non-protein amino acids also clearly indicates prebiotic synthesis. Further, the remarkable similarity in the amino acid abundance of the exterior and interior suggests that the terrestrial organic contamination was minimal.

More confirmation is provided by the equal abundance of D- and L-enantiomers of protein amino acids such as alanine, aspartic acid and glutamic acid in both the exterior and the interior. A similar study of the Mighei meteorite showed a striking L-enantiomer predominance of protein amino acids in its exterior portion, which diminished towards the interior of the

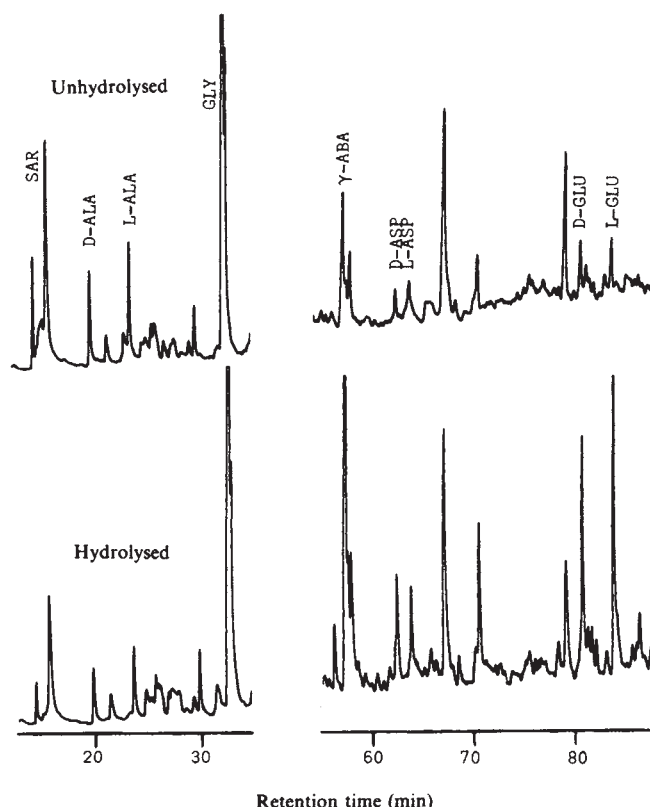


Fig. 2 Gas chromatograms of amino acids (N-TFA-isopropyl esters) of the interior portion of the Yamato meteorite 74662.

meteorite. The non-protein amino acids, however, did not show a difference throughout the exterior to interior portions¹⁵.

The Yamato meteorite contains amino acids similar to those in the Murchison and Murray meteorites. All three are type II carbonaceous chondrites. The abundance of amino acids are also of the same order of magnitude. Glycine, the most abundant amino acid, is 34 nm in the Yamato, 81 nm in the Murchison^{8,10,11}, and 40 nm in the Murray¹¹. Another common feature is the marked increase in the yields of aspartic acid and glutamic acid (both dicarboxylic amino acids) after acid hydrolysis. The Murchison and Murray meteorite also have aspartic acid and glutamic acid in very small quantities in the unhydrolysed portion¹¹. This resemblance minimises the possibility that these amino acids in the unhydrolysed portion of the Yamato meteorite had been washed out by water on the surface of bare ice because dicarboxylic amino acids are anionic under a neutral pH. The observation of the recovered body of the Yamato meteorite indicated that about half of the fusion crust was lost by weathering¹⁴. Nevertheless, the meteorite does not seem to have been significantly altered by weathering. It seems that only the portion of the crust, physically broken from the

parent body, was lost during its period on Earth.

The Allan Hills carbonaceous chondrite, also from Antarctica, exhibits an equal abundance of the D- and L-enantiomers in the exterior and interior¹⁶. Although the amino acid concentration was much lower in the Allan Hills meteorite than in the Yamato meteorite, these two Antarctic carbonaceous chondrites seem to be the least contaminated of the meteorites we have examined so far.

Since the fall of the Murchison meteorite, information on organic compounds indigenous to carbonaceous chondrites has revealed the processes of primordial organic synthesis. Studies of Antarctic carbonaceous chondrites, such as the Yamato 74662 which contains organic material of nearly the same concentration level as that of the Murchison meteorite, provide additional information useful for the understanding of extra-terrestrial organic material.

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Quantification of monocarboxylic acids in the Murchison carbonaceous meteorite

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Although the nature and origin of carbon and its compounds in meteorites have been reviewed by Hayes¹ and Nagy², much of our understanding of carbon and its compounds in carbonaceous chondrites remains speculative. Evidence for the extraterrestrial nature of the solvent-soluble organic matter was first found in the Murchison carbonaceous chondrite³; this material in the Murchison meteorite and in other meteorites has been identified as amino acids and precursors^{3–10}, monocarboxylic acids¹¹, dicarboxylic acids¹², hydrocarbons^{6,13,14}, amines¹⁵, and other organic compounds^{16–20}. The data suggest a chemical synthesis from simple precursors. Although the mechanisms by which these organic compounds were formed are unknown, theories (such as Fischer-Tropsch Type (FTT)²¹ and discharge reactions²²) have been proposed to account for their presence. Knowledge of the distribution of isomers in these chondrites is essential for understanding the mechanism of synthesis, and we report here the quantification of some of the branched- and straight-chain isomers of the monocarboxylic acids extracted from the Murchison meteorite.

The successful use of deuterated analogues for the quantification of amino acids²³ led to the selection of this approach for the quantification of the monocarboxylic acids. The deuterated and undeuterated monocarboxylic acid solutions for calibration and spiking purposes consisted of the 11 acids listed in Table 1. The deuterated analogues were prepared by the method of Pfeffer and Silbert²⁴.

A 1.084-g sample of finely crushed Murchison (interior) was refluxed for 3 h in a KOH-methanol solution (0.5g : 8 ml). After cooling to room temperature, the methanolic solution with some suspended solids was decanted into a Teflon test tube and the residue was washed twice with 5-ml portions of distilled water. After the combined solutions had been centrifuged, the supernatant was transferred to a 25-ml round-bottomed flask and the solvents were removed under reduced pressure. The resulting residue was acidified to pH 1 by adding concentrated hydrochloric acid dropwise, while cooling the flask in an ice-water bath. The aqueous mixture was filtered to remove inorganic salts and the filtrate collected in a screw-cap vial. The inorganic salts were rinsed with 3.0 ml of diethyl ether and collected in the same vial that contained the aqueous acid solution. After shaking for 1 min, a sample of the ether phase was analysed by gas chromatography using a 46-m-long stainless steel column with an i.d. of 0.05 cm; the column was coated with UCON 550-H₃PO₄. Comparison of this gas chromatogram with that of a standard solution of deuterated carboxylic acids prescribed the quantity of the deuterated solution to be added to the meteorite extract in order to have approximately a 1:1 correspondence in molar concentration between the carboxylic acids in the meteorite sample and their deuterated analogues. After adding the solution of deuterated acids and intimate mixing, the ether layer was separated from the aqueous phase and the latter was extracted three times with 1-ml portions of diethyl ether. The combined ether extracts were dried over sodium sulphate, filtered and concentrated to a small volume (~50 µl) for analysis by combined gas chromatograph-mass spectrometry (GC-MS).

Table 1 Monocarboxylic acids in Murchison meteorite

Compound	Abundance ($\mu\text{mol g}^{-1}$)	$N/\Sigma Br$
Ethanoic	1.03 ± 0.06	—
Propanoic	1.83 ± 0.03	—
2-Methylpropanoic	0.50 ± 0.06	0.76
Butanoic	0.38 ± 0.02	
3-Methylbutanoic	0.09 ± 0.02	0.57
2-Methylbutanoic	0.12 ± 0.02	
Pentanoic	0.12 ± 0.03	0.86
4-Methylpentanoic	0.07 ± 0.02	
Hexanoic	0.06 ± 0.01	
Heptanoic	0.03 ± 0.01	
Octanoic	0.01 ± 0.002	

To determine the effectiveness of this procedure to extract the monocarboxylic acids from the sample, the solid meteorite residue was again pulverised and re-extracted by the procedure described. No monocarboxylic acids were found in the second extract, which strongly suggests that the first extraction process had completely removed these acids from the sample. Therefore, the values for the monocarboxylic acids (Table 1) are good estimates of the abundance of these compounds in this sample of the Murchison meteorite. Satisfactory procedural blanks accompanied all the experiments.

An area of concern is the possible degradation of these saturated monocarboxylic acids during extraction. However, the potassium hydroxide and refluxing methanol involved, are also used in a procedure to identify these acids²⁵ and are not known to degrade them²⁶. It is reasonable to assume that the saturated monocarboxylic acids should survive a similar treatment.

Our procedure was similar to that used previously but with slight modification²³. Mass spectra were acquired every 4.2 s over the mass range 12–400 throughout the GC run. Identity of the individual components was established by evaluation of the mass spectra obtained for each component eluting from the GC. Quantification was accomplished by selection of mass spectral ions of the corresponding deuterated and undeuterated analogues of each monocarboxylic acid and measurement of the area ratio of each m/e pair within a given gas chromatographic peak. The results of this measurement from the deuterium-spiked Murchison meteorite extract were then compared with standard calibration curves obtained in an analogous fashion from standard mixtures. The quantification data for the monocarboxylic acids reported in Table 1 were obtained in this way.

As an extraterrestrial chemical synthesis seems to be responsible for the presence of the monocarboxylic acids in the Murchison meteorite, a similar distribution of decreasing concentration with increasing molecular weight would be expected to that found for the amino acids⁹. Such a distribution for the monocarboxylic acids was not obvious from the data previously reported¹¹. In that study, the GC peak heights (and area) for propanoic and hexanoic acids appeared to be equal, suggesting equal concentrations of these two compounds. In reality, propanoic acid is 30 times more abundant than hexanoic acid (Table 1). Gas chromatogram peak areas do not adequately reflect the distribution of components, as these results could be an artefact of the analytical procedure used. For example, the distribution of the acids previously observed could have been skewed by the differences in partition coefficients of these acids in the solvents utilised. Thus, the quantification data presented in Table 1 reflect more accurately the monocarboxylic acid distribution in that the deuterated analogues added to the meteorite extract during the isolation procedure would be subjected to the same analytical skewing.

The monocarboxylic acids are present in the Murchison meteorite in higher concentration than are the amino acids, which are the only other group of compounds that have been rigorously quantified^{5,10,23}. The concentration of propanoic acid ($1.83 \mu\text{mol per g}$ of meteorite), the most abundant monocarboxylic acid, is ~ 58 times greater than the most abundant

amino acid, glycine ($0.041 \mu\text{mol g}^{-1}$)²³ found in aqueous extracts of the Murchison meteorite. We also note a general decrease in concentration of the monocarboxylic acids as the number of carbon atoms in these acids increased, a trend similar to that previously noted for the amino acids⁹. This supports the hypothesis that monocarboxylic acids, like amino acids, are present as the result of an extraterrestrial abiotic synthesis. Table 1 shows that nearly equal concentrations of each branched- and straight-chain isomer with the same carbon number are present in the sample.

Miller²⁷ showed that formic, acetic and propanoic acids could be synthesised when a primitive-Earth-type reducing atmosphere was subjected to a spark discharge. Similarly, C_2 – C_{12} monocarboxylic acids have been produced when a methane–water mixture was exposed to a semicorona discharge²⁸. In the discharge experiment monocarboxylic acids with carbon numbers less than six were present both as straight- and branched-chain isomers, but for those acids with carbon numbers of six or greater, only the branched-chain isomers were observed²⁸. Since the Murchison meteorite contains straight-chain monocarboxylic acids with carbon numbers greater than five (hexanoic, heptanoic and octanoic acids) a re-evaluation of the laboratory simulation experiment is warranted. An alternative mechanism (FTT) has been proposed as the possible pathway to the organic compounds in meteorites²¹. Theoretical consideration of the proposed mechanism for the FTT process predicts a high concentration of straight-chain isomers, with $N/\Sigma Br > 1$, for any given carbon number (N , straight-chain isomer concentration; Br , branched-chain isomer concentration). Recent FTT experimental studies in which monocarboxylic acids were synthesised²⁹ support this prediction.

Although Leach *et al.*²⁹ were not able to quantify the C_2 – C_6 monocarboxylic acids which would have allowed a direct comparison of their results with those reported in Table 1, they did report quantification of the C_7 – C_{18} monocarboxylic acids that were synthesised by the FTT process. Straight-chain isomers were observed to predominate over the branched with $N/\Sigma Br$ ratio falling between 1.7 and 5.

In contrast, the predominance of straight- over branched-chain isomers is not observed for the isomeric monocarboxylic acids found in the meteorite extract ($N/\Sigma Br < 1$, Table 1). Moreover, this study and previous work¹¹ indicate that other branched-chain isomers are present. If these were included in the quantification, the value of $N/\Sigma Br$ would be further decreased. Thus, if the monocarboxylic acids present in the Murchison meteorite are the original, unchanged, extraterrestrially synthesised compounds, its isomer distribution is not consistent with that of the FTT process. Thus the FTT process, conducted in reported experimental conditions²⁹, is not apparently singly responsible for the formation of the acids found in the Murchison meteorite. If a two-step mechanism is envisaged, a primary FTT reaction followed by a secondary partial equilibration²¹, then the effect of the equilibration step on product distribution deserves careful examination. It has been predicted on theoretical grounds³⁰ that there would be little difference in concentration between straight- and branched-chain isomers of the short-chain (C_2 – C_8) monocarboxylic acids formed inorganically in equilibrium conditions. The meteorite results reported here apparently support this prediction with regard to isomer distribution. Our results are consistent with the production of the monocarboxylic acids by a naturally occurring chemical synthesis and support the chemical evolution theory.

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Experimental evidence of rare earth element immobility in greenstones

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Significant petrogenetic importance has recently been placed on the Nd isotopic composition and variable rare earth element (REE) content of basalts, although the effect of alteration on the REE has not been fully investigated. REE analyses of oceanic^{1–6} and ophiolitic basalts^{7,8} suggest that the light REE (La, Ce, Nd, Sm, Eu) are mobile during basalt–seawater interaction at relatively low temperatures. However, the behaviour of the trace and rare earth elements during greenstone formation at higher temperatures is less well understood^{9–15}, primarily because of uncertainties regarding the composition of the unaltered precursor, temperature, pressure and effective water/rock ratio. In an attempt to overcome these uncertainties we have analysed basalt glass subjected to 10 individual basalt–seawater interaction experiments. In all these experiments the REE are found to be immobile at temperatures of 150–350 °C even when the basalt is totally altered to clay. Furthermore the data suggest that the minor fluctuations observed in the light REE content of some greenstones^{9,10} may result from a low temperature overprint due to retrograding of the greenschist facies basalts.

Details of the experimental, analytical and sampling procedures used in this study have been described elsewhere^{8,15,16}. Briefly, experiments were performed at temperatures from 150 to 350 °C, 500 bar and water/rock mass ratios of 10–125 using Dickson hydrothermal equipment¹⁶, and REE were analysed by instrumental neutron activation analysis (INAA). Figure 1 shows the REE composition of the fresh basalt glass (a) and experimental alteration products relative to chondrite (b–e).

The glassy tholeiite (that is, unaltered precursor) has a light REE depleted profile with a detectable Eu anomaly—features characteristic of many ocean-floor basalts. All of the experimentally altered basalts have similar REE profiles, although

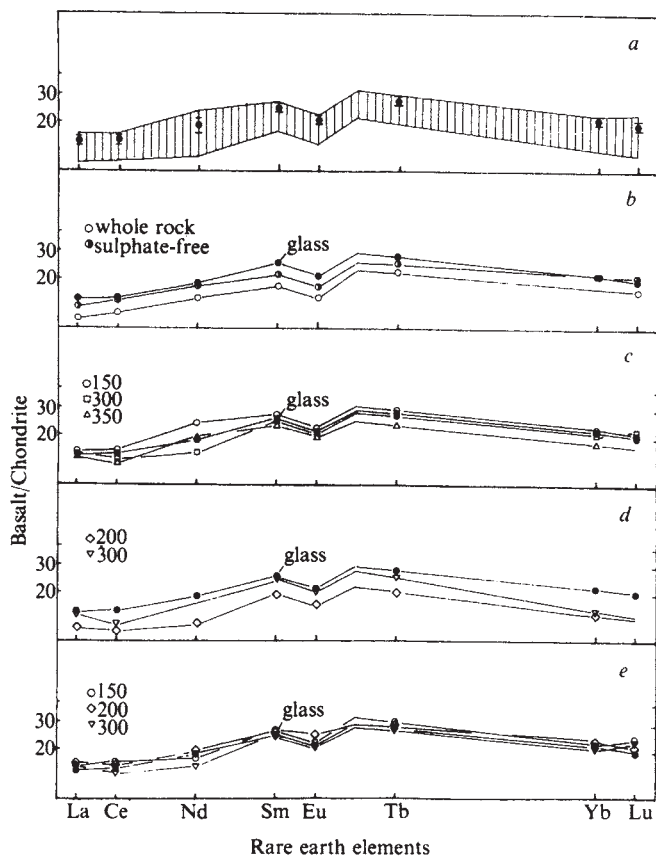


Fig. 1 Chondrite normalised REE patterns of experimentally altered basalt and the unaltered precursor. The figure includes the REE abundance profile of a, the fresh glass (●) with error bars and the total range in REE produced by alteration of this glass at 150–350 °C and a water/rock ratio (W/R) of 10–125; b, the whole rock altered at 300 °C and a W/R 125 before and after correction for 20% modal anhydrite; c, the whole rock altered at 150–350 °C and a low W/R of 10; d, the whole rock altered at 200–300 °C and a higher W/R ratio; and e, the silicate fraction (chlorite + smectite) altered at 150–300 °C and a W/R of 62 (anhydrite was removed by repeated washings in deionised water¹⁵).

they differ in their absolute REE content. We believe this to be a dilution effect caused by the presence of anhydrite, a phase (CaSO₄) which precipitated during the experiments¹⁵. For example, anhydrite constituted approximately 20% of the 'bulk' alteration from interaction at 300 °C and a water/rock ratio of 125. Anhydrite contains an insignificant amount of REE, so its presence simply and effectively dilutes the REE abundances of the alteration products as a whole. Thus correcting for the presence of anhydrite, a REE profile develops which is identical to that of the unaltered basalt (Fig. 1b). This is highly significant since the basalt was entirely transformed to a mixed layer chlorite–smectite phase. This was the case for all silicate fractions (altered basalt minus anhydrite)¹⁵ separated from the altered solids from each experiment. Thus, for the duration of these experiments, REE were conserved and in general revealed a solubility similar to previously defined conservative species, such as Co, Cr, Ni, Al, V and Ti (W.S. and M.J. Mottl, unpublished).

Hydrothermal alteration of the oceanic crust and greenstone formation apparently occurs at temperatures, pressures, and water/rock ratios analogous to those used in these experiments. The immobility of REE in greenstones from the Point Sal⁸ and Troodos^{11,19,20} ophiolites, and the Mid-Atlantic¹⁰ Ridge compares favourably with the data presented here. Similarly, amphibolites²¹ and chlorites²² from the seafloor exhibit REE abundances typical of oceanic basalts. In the case of the chlorites

it was proposed that they represent disrupted fragments of submarine greenstones²². This conclusion is certainly compatible with the results of the present investigation which confirm the relative immobility of REE during hydrothermal processes (Fig. 1), hence, their use as a 'fingerprinting' tool to decipher origins of recent and Archean^{9,23} greenstones.

However, it is difficult to reconcile the fluctuations in light REE content of some greenstones with the experimental data. Because the light REE are more mobile at low temperatures than at 150–350°C the apparent mobility of the light REE in rocks with greenschist facies mineralogy may partly reflect retrograde reactions. Such reactions would superimpose a low temperature overprint on the greenstone thus modifying the mineralogy and chemistry of the rock.

Massive volumes of seawater must be involved in retrograde processes if these events are to substantially modify the REE content of basalt. Furthermore, that LREE are specifically enriched during this event suggests ionic size-charge relationships may be important if not restrictive to secondary REE enrichment. Certainly this is true for post-greenstone enrichment of K and Rb. These species are apparently incorporated in cation exchange sites of clay minerals (smectite, mixed layer chlorite-smectite, chlorite) in greenstones. Sediments found within the Troodos ophiolite have a La, Ce, and Nd content 10–100 times that of basalt⁷. Similarly, clay rich umbers⁷ (ferromanganous oxyhydroxides and silicates) concentrate the light REE (except Ce) relative to basalt. Thus the light REE can be enriched in clay minerals within greenstones provided adequate exposure to seawater is maintained for prolonged periods of time (>1 Myr). This is particularly noticeable in basalts found within or adjacent to the discharge zones of the Troodos submarine geothermal system⁷. The basalts are characterised by pronounced negative Ce anomalies and enhanced La and Nd contents, whereas, zeolite and greenschist facies^{11,20} basalts isolated from abundant seawater show no such REE mobility.

The effect of availability and accessibility of seawater and REE mobility is also amply illustrated in the Point Sal⁸ ophiolite, California. At Point Sal the upper smectite bearing lavas have been exposed to seawater for ~15 Myr before deposition of a sedimentary horizon primarily consisting of chert²⁶. Thus, these volcanic flows were actively and effectively altered by seawater for approximately 15 Myr before being isolated. The light REE in these lavas were mobilised and are now characterised by negative Ce anomalies⁸. Similar anomalies have been observed in seawater²⁷, marine precipitates^{7,27}, and basalts^{2,4,7} exposed to low temperature alteration or retrograde reactions. However, at a stratigraphically lower level in the Point Sal ophiolite; greenstones show little or no change in REE abundance even though these rocks are thoroughly altered and characterised by abundant epidote and quartz (epidosite)⁸. We believe that the apparent absence of REE mobility during greenschist facies metamorphism results from limited exposure of these rocks to fluids (seawater) at low temperatures. Vein mineralisation during incipient alteration may have effectively reduced the permeability of these rocks to the point of not permitting access of seawater subsequent to the primary alteration event.

Variations in the light REE content of greenstones relative to an inferred unaltered precursor have been noted^{9,10,24}. These changes may in part reflect alteration effects subsequent to the metamorphic event which initially transposed the basalt to greenstone. The suggestion that greenstones are modified chemically subsequent to their formation is supported by the light REE enrichment noted and the trace element abundances reported for these rocks. It has been shown experimentally that K is leached from basalt¹⁵ reacted with seawater at temperatures $\geq 150^\circ\text{C}$; whereas, at lower temperatures the reverse is true, that is, K is removed from seawater and enriched in the altered basalt²⁵.

The above discussion emphasises that metamorphic minerals produced during greenstone formation immobilise the REE and that changes in the light REE may result from retrograde effects. There remains the problem of spilites and greenstones with

modified ΣREE contents. The origin of these rocks is clearly complex, although Sun and Nesbitt⁹ and Hellman and Henderson¹³ attribute the consistent change in REE profile to alteration processes. However, the REE content of an altered rock is influenced by, (1) the chemistry of the unaltered precursor, (2) the extent of igneous fractionation²⁸, and apparently (3) the degree of retrograde reactions. Thus the REE chemistry of these rocks^{9,13} probably represents a complex interplay of primary and secondary processes, and their origin is at best equivocal.

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A new date for *Ramapithecus*

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Recent work in the Middle Siwalik rocks of the Potwar Plateau in northern Pakistan is doing much to further our understanding of Miocene mammalian evolution, in particular the evolution of *Ramapithecus* and *Sivapithecus*, hominoids considered important for human ancestry¹. It is crucial to the understanding of hominoid evolution to place the fossils in as precise a chronologic framework as possible. As there is a lack of material suitable for radiometric dating, it is necessary to rely on the correlation of the magnetic stratigraphy associated with the Siwalik faunas to the calibrated magnetic polarity time scale (MPTS). Initial age estimates by Barndt *et al.*² were based on reconnaissance level palaeomagnetic sampling. The more detailed sampling presented here suggests a revised correlation of the local magnetic stratigraphy to the time scale and hence a revised age estimate for *Ramapithecus*: 8 Myr now seems more likely than the previous estimate of 9 Myr.

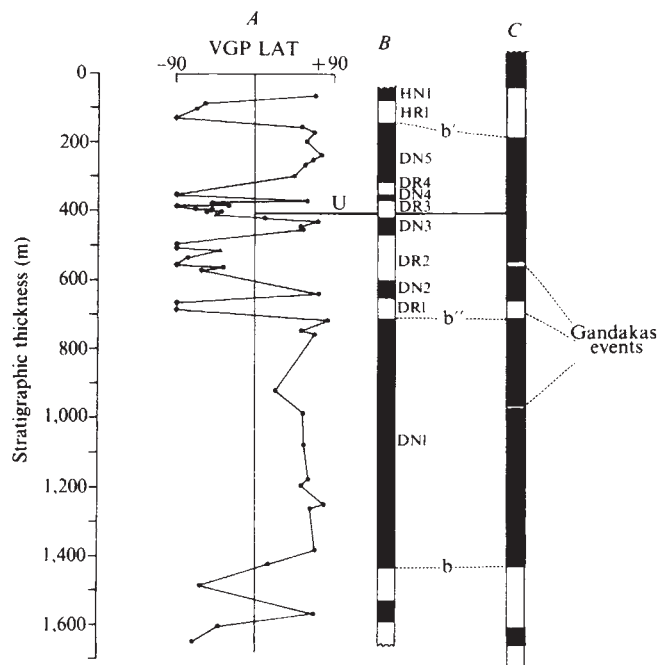


Fig. 1 Column A, VGP latitudes calculated from site means of the Ratha Kas section¹. Class A, B and C data (see text) are denoted by circles, triangles and diamonds respectively. Column B, Block diagram of section in (A), black is normal polarity, white is reversed. Magnetozones D and H stand for Dhurnal and Hasal chronostratigraphic units¹². N and R stand for normal and reversed respectively. Column C, Part of the magnetic stratigraphy of Barndt *et al.*² correlated to section shown in (A) and (B) by means of stratigraphic marked bed U.

Barndt *et al.*² established a local magnetic stratigraphy which was correlated to the MPTS based on various faunal constraints and later showed it to be consistent with a dated bentonite³. The bulk of the hominoid material was thus estimated to be around 9 Myr old. In the work described here, part of the magnetic stratigraphy of Barndt *et al.*² was examined in great detail, originally to precisely define an isochron within the sediments that could be used for lateral correlation of hominoid levels. However, as a result of this more detailed work it became clear that the original identification of the magnetic reversal sequence needed revision.

The location, geological setting and stratigraphy are described in detail elsewhere⁴. Briefly, the strata exposed on the northern flank of the Soan Synclinorium are cut by rivers flowing approximately perpendicular to strike. The sampling technique used was similar to that described by Johnson *et al.*⁵ which is ideally suited for Siwalik sediments. Sampling of river channel exposures resulted in the section shown in Fig. 1. Each site consists of three to five samples and the sites were placed in a stratigraphic section constructed by members of the joint GSP-Yale University field party. The section presented in Fig. 1 consists of 81 sites in 1,600 m for an average site density of about 1 in 20 m. The actual site density is dependent on the relative abundance of sandstone (unsuitable for palaeomagnetic purposes) and the finer grained units, and thus varies over the thickness of the section. The maximum separation is estimated to be about 100 m and the minimum separation is about 1.5 m.

To allow unambiguous correlation of the magnetostratigraphic section of the Kaur area, a major sandstone unit, referred to here as the 'U' sandstone, was carefully traced in the field from section to section. The magnetic stratigraphy presented here can thus be precisely correlated to the sections of Barndt *et al.*².

An extensive study of the magnetic mineralogy of Middle Siwalik rocks has been completed, including a conglomerate test

coupled with rock magnetic studies which will be presented elsewhere. It is reasonable to assume that the primary remanence of the rocks is carried by haematite, was acquired during or soon after deposition, and can be isolated by thermal demagnetisation. After thermal demagnetisation at 550 °C, site means were calculated using Fisher statistics⁵. Sites found to be statistically significant by Watson's test for randomness⁷ (for a mean of three sample directions of unit length, the resultant vector length must be ≥ 2.62) after thermal cleaning are designated Class A. Statistically significant sites after a.f. demagnetisation at peaks of 300 Oe are Class B, useful primarily when the specimens disintegrate on heating. Class C are sites with two specimens showing reversed directions in good agreement where the third is either lost, crumbled or widely deviant. Class D are samples showing a strong distribution to the South and are thought to be possibly reversed. Only Class A, B and C data are used here. Class A and B site means were converted to virtual geomagnetic pole (VGP) latitudes.

In Fig. 1, Classes A, B and C are represented by circles, triangles and diamonds, respectively. In some cases sites showing a statistically significant normal cluster after a.f. treatment became reversed after thermal cleaning. To ensure the best quality palaeomagnetic data, only Class A data are used for normal polarity information as there is mounting evidence to indicate that a.f. demagnetised specimens have not been properly cleaned. Each labelled magnetozone of Fig. 1 has been substantiated in at least three stratigraphically correlatable sections in the Kaur area, strengthening the assumption that they represent palaeomagnetic field behaviour.

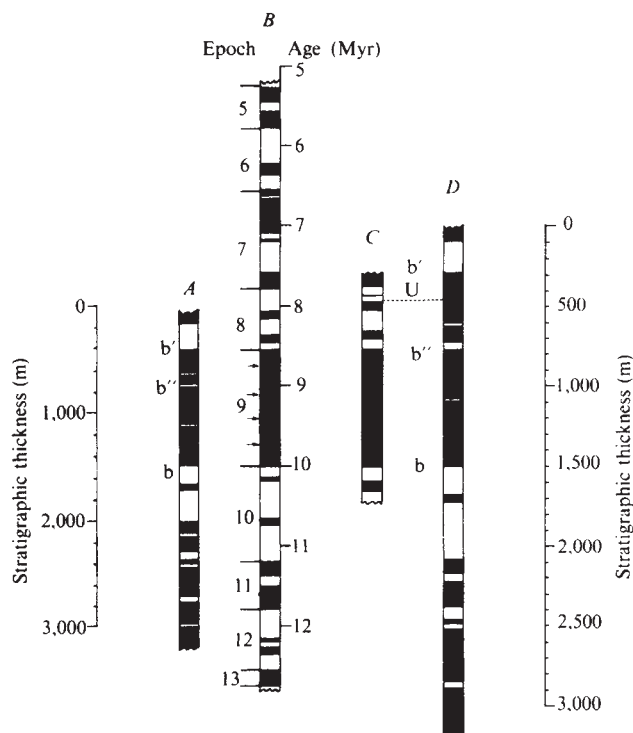


Fig. 2 Correlation of the magnetic stratigraphy to the magnetic polarity time scale. Column A, Previous correlation to the time scale of the Barndt *et al.*² section. Column B, The revised dates given by Mankinen and Dalrymple¹³ for the time scale of LaBrecque *et al.*¹⁴. Column C, The section presented in this paper drawn to scale at right assuming DN1 (see Fig. 1) is Epoch 9 of the time scale. Column D, The section in (A) redrawn at the same scale as the section in (C). The stratigraphic marker bed U allows unambiguous stratigraphic correlation of the Barndt *et al.*² section in (D) and the more densely sampled section of (C). The bulk of the primate material has been found in close association with the U marker bed and thus is about 8 Myr old.

The magnetic stratigraphy of Barndt *et al.*² established the existence of a long zone of dominantly normal polarity (b to b' of column C of Fig. 1) which was apparently punctuated by several reversed events known as the 'Gandakas Events'. The Barndt *et al.*² section was correlated to the MPTS as shown in Fig. 2A based on faunal constraints and visual correlation. The magnetic stratigraphy in Fig. 1 represents a more densely sampled section of the same strata and it is clear that the Gandakas Events represent more reversed time that was previously supposed.

Several assumptions must be made to make a useful correlation of the magnetic polarity stratigraphy to the MPTS. These include: (1) an estimate of the range of ages that the section could encompass; (2) that for a given lithofacies, when averaged over a large stratigraphic thickness, thickness is linearly proportional to time; (3) the section is densely sampled enough to assume that it approximates field behaviour; and (4) the magnetic remanence of the rock was acquired shortly after deposition and represents a record of the magnetic field at the time of formation of the rock. These assumptions are not always valid and great care must be taken to substantiate them whenever possible.

Both Barndt *et al.*² and the magnetic stratigraphy of this paper are bounded by the following palaeontological criteria. A maximum age for the bottom of the section is given by the sudden appearance of the extinct equid, *Hipparion*, within the Khaur normal zone (DN1 of Fig. 1). The earliest Old World *Hipparion* has been dated⁸ at 12.5 Myr, and the Indian *Hipparion* is probably younger (B. MacFadden, personal communication). A minimum age for the top of the section is given by the conspicuous absence of hippopotamus, *Hexaprotodon*, which occurs suddenly and abundantly in the Upper Siwaliks. The oldest hippopotamus in the Siwaliks is found in the Rhotas section in the Gilbert/Epoch 5 boundary. The absence of *Hexaprotodon* (John Barry, personal communication) in the section presented here and in that of Barndt *et al.*² therefore suggests that the top of the section is probably older than about 5 Myr.

Based on the more detailed section presented here it seems unlikely that the Gandakas Events (Fig. 1C) are all within Epoch 9 because (1) in the more detailed section shown in Fig. 1, the reversed zones represent a large stratigraphic thickness and it is unlikely that such long reversed zones would be unresolvable in the seafloor spreading record, and (2) the four events or intensity fluctuations discussed by Blakely¹⁰ and Cande and Labrecque¹¹ are evenly spaced within anomaly 5 (Epoch 9) and the reversed zones in Fig. 1 are tightly grouped. A better correlation of the magnetic stratigraphy of the Khaur area, based on the work presented here, is shown in Fig. 2C where the vertical scale is expanded by assuming the two upper Gandakas Events to comprise Epoch 8. Epoch 9 is thus b to b' of Fig. 2. Figure 2D shows the Barndt section redrawn to the same scale as Fig. 2C. Clearly the overall correlation is much improved and there is now a reasonable fit to the time scale from the top of Epoch 8 to the bottom of Epoch 11. Below Epoch 11 there seems to be a dominance of normal polarity which seems to correlate with an increase in red pigmentation. Recent efforts using thermal demagnetisation techniques have improved the fit below Epoch 11 dramatically (to be presented elsewhere).

Fig. 1 suggests three short normal subzones, DN2-DN4, within the dominantly reversed zone. Since the normal subzones occur in correlated sections of different rock types and persist after thermal demagnetisation, these are thought to be real. The uppermost zone, DN4, was ignored in the correlation process because in most sections it is represented by only one site, even when sampled at 1.5 m intervals and is therefore likely to be genuinely short and thus undetectable from the seafloor spreading record. Accepting that the long normal DN1 is Epoch 9, and that thickness is approximately linearly related to time over this interval, then the best match to the time scale (that requiring the least condensing or stretching of the section) is that adopted in Fig. 2C. Thus the youngest of the three normal subzones DN4 within the reversed zone seems to be a previously undetected

normal event within Epoch 8.

The results presented here emphasise the importance of adequate specimen demagnetisation and sampling density. Based on the previous reconnaissance level work the correlation appeared quite reasonable, but this detailed work has revised the age scale significantly. The bulk of the hominoid localities in the Khaur region fall within a narrow band associated with the U stratigraphic marked bed and a date of 8 Myr is apparently much more reasonable than the previous 9 Myr.

Even with adequate sampling density, palaeomagnetic stratigraphy in Middle Siwalik rocks is more uncertain than in rocks younger than about 5 Myr because (1) the time scale itself is less well defined and (2) the rocks have undergone prolonged diagenetic alterations (such as red pigment formation) which often obscures the original magnetisation of the rock. For these reasons, work is in progress to extend the magnetic stratigraphy pattern into younger rocks in order to increase the reliability of the correlation and the inferred ages.

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Involvement of hydrogen sulphide in the degradation of parathion in flooded acid sulphate soil

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There is increasing evidence that certain pesticides, resistant to degradation in aerobic systems, undergo fairly rapid degradation in anaerobic ecosystems such as flooded soil^{1,2}. After flooding, the soil components NO₃⁻, Fe³⁺, Mn⁴⁺ and SO₄²⁻ are reduced almost in a thermodynamic sequence with a corresponding shift in favour of anaerobic microorganisms³. Although anaerobic microorganisms have been implicated directly in the degradation of some pesticides in anaerobic environments^{1,2}, there is circumstantial evidence for chemically catalysed reactions, involving, for example, the iron redox system⁴ or reduced iron porphyrins⁵. However, the importance of this and other dominant redox reactions in the behaviour of pesticides in a flooded soil is not well understood. We report here the involvement of hydrogen sulphide, the end product of sulphate reduction, in the degradation of parathion in a flooded acid sulphate soil.

Portions (10 g) of laterite (pH, 6.3; organic matter, 2.88%; S, 0.01–0.03%) and acid sulphate (pH, 5.2; organic matter, 5.52%; S, 0.2%; known as pokkali) soils were reduced by flooding with 10 ml of distilled water for 60 d in screw-capped glass tubes (30×120 mm, 30 ml capacity). The 60-d flooded soils were shaken with 160 µg of parathion in acetone for 30 min. The soils, flooded immediately before equilibration with parathion, served as oxidised soils. After 30 min of equilibration, the residues of parathion and its metabolites were extracted from the soils with acetone–sodium sulphate–hexane and the hexane layer was analysed by gas-liquid chromatography using a phosphorus-specific photometric detector. To measure soil reduction due to flooding, the 60-d flooded soil sample in each tube was transferred to a beaker and redox potentials were assayed immediately with a compound platinum–calomel electrode as described before⁶. The variations in soil potential in five replicates were less than 5% by this method. Redox potentials decreased from +260 to –140 mV in laterite soil and from +200 to –200 mV in acid sulphate soil after 60 d of flooding, confirming the reduction of the soil.

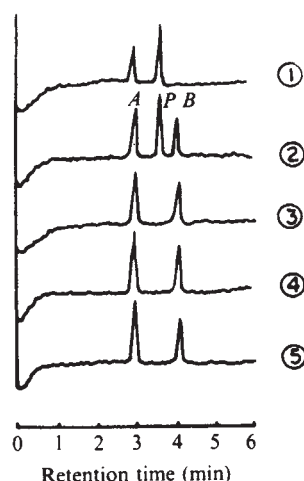


Fig. 1 Gas chromatograms of the products of reactions between: 1, parathion and prereduced laterite soil; 2, parathion and pre-reduced acid sulphate (pokkali) soil; 3, aminoparathion and pre-reduced pokkali soil; 4, aminoparathion and sodium iodide; 5, aminoparathion and hydrogen sulphide. A, aminoparathion; P, parathion; B, desethyl aminoparathion. The soils were pre-reduced by flooding for 60 d before equilibration with parathion (1 and 2) and aminoparathion (3).

After 30 min of equilibration with 60-d flooded soils, the concentration of parathion decreased to 13.8% of the original level in the acid sulphate soil and to 52.1% in the laterite soil. During the same period, 92–95% of added parathion was recovered from the oxidised soils. The gas chromatograms of the residues (Fig. 1) showed that the rapid destruction of parathion in equilibration with reduced soils led to the formation of one compound (peak A) in laterite soil and two compounds (peaks A and B) in acid sulphate soil. Based on chromatographic characteristics (retention time, 2.9 min; R_F , 0.35) and its positive reaction to palladium chloride spray, compound A was identified as aminoparathion, a major product of parathion metabolism in soils and in microbial cultures⁷.

To characterise compound B, authentic aminoparathion was equilibrated with 60-d flooded pokkali soil for 30 min. A gas chromatogram of the residues showed the formation of a compound with the same retention time as that of compound B

(Fig. 1), which we therefore presume was formed after aminoparathion.

We investigated whether compound B was a dealkylation product of aminoparathion as follows. Desethyl aminoparathion was prepared by a reaction between aminoparathion and sodium iodide, essentially as described by Miyamoto *et al.*⁸. Portions of 20 ml each of 0.05 M solutions of aminoparathion and sodium iodide in acetone were refluxed at $65 \pm 1^\circ \text{C}$ in a water bath for 3 h. This chemical reaction produced only desethyl aminoparathion, which had characteristics (retention time, 4 min; R_F , 0.47; positive reaction to palladium chloride spray) identical to those of compound B. Thus compound B was identified as desethyl aminoparathion. This is the first report of the formation of a dealkylation product in the degradation of parathion in soils. There is evidence that the degradation of related organophosphates, methyl parathion and fenitrothion, by *Bacillus subtilis* leads to the formation of their respective amino analogues and dealkylation products, desmethyl aminoparathion and desmethyl aminofenitrothion⁸.

It is interesting that desethyl aminoparathion was generated from added parathion or aminoparathion only by reduced pokkali soil and not by laterite soil. Unlike laterite soil, pokkali soil is rich in organic matter and sulphate and is classified as acid sulphate soil⁹. Sulphate reduction leading to the formation of hydrogen sulphide is important in flooded soils, especially rich in organic matter and sulphate³. Studies in our laboratory (R. C. Ray, unpublished results) showed that after the redox potential of the flooded pokkali soil had dropped to –160 mV—a potential favourable for sulphate reduction¹⁰—there was significant production of hydrogen sulphide and decrease in sulphate content. In contrast, the formation of sulphide in laterite soil was negligible. These results confirmed the substantial formation of sulphide in pokkali soil when it is flooded.

To investigate the involvement of hydrogen sulphide in the dealkylation of aminoparathion, hydrogen sulphide evolved by reaction between sodium sulphide and dilute hydrochloric acid was bubbled through a solution containing 1,000 µg of aminoparathion in 2 ml of acetone and 25 ml of distilled water. The product of the reaction was extracted with acetone–sodium sulphate–hexane and analysed by gas-liquid chromatography. Only one product was detected and that had a retention time similar to that of desethyl aminoparathion and compound B formed by the reaction of parathion and aminoparathion with reduced pokkali soil (Fig. 1). When parathion was treated similarly with hydrogen sulphide, aminoparathion and desethyl aminoparathion were not formed. This implies that hydrogen sulphide formed readily in flooded pokkali soil was, at least in part, responsible for the formation of the dealkylation product from parathion through aminoparathion. These findings indicate that chemical degradation of pesticides mediated by the products of anaerobic decomposition of inorganic and organic soil components can be more common and widespread in anaerobic ecosystems than hitherto believed.

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Intermodal matching by human neonates

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Normal human adults judge two identical objects to have the same shape even when they are perceived through different modalities, such as touch and vision. The ontogenesis of man's capacity to recognise such intermodal matches has long been debated. One hypothesis is that humans begin life with independent sense modalities and that simultaneous tactual and visual exploration of shapes is needed to learn to correlate the separate tactual and visual sense impressions of them¹⁻³. A second hypothesis is that the detection of shape invariants across different modalities is a fundamental characteristic of man's perceptual-cognitive system, available without the need for learned correlations⁴⁻⁷. Recent research has shown that 6-12-month-old infants can recognise certain tactual-visual matches⁸⁻¹¹. However, such data cannot help resolve the classic theoretical debate. Infants of this age repeatedly reach out and touch objects they see, and such simultaneous bimodal exploration presumably offers ample opportunity for learning to correlate tactual and visual sense impressions. The experiments reported here show that humans can recognise intermodal matches without the benefit of months of experience in simultaneous tactual-visual exploration. We demonstrate that 29-day-old infants can recognise which of two visually perceived shapes matches one they previously explored tactually, thus supporting the second hypothesis listed above.

For our assessment of intermodal matching, we adapted a paradigm used to test infant memory¹². We began with a brief familiarisation period during which the infant tactually explored an object. Next, the infant was shown a pair of visual shapes, only one of which matched the tactual stimulus. Visual fixation to the matching versus non-matching shape was then recorded. Three experimenters were used to ensure objectivity of the results. One experimenter selected the tactual shape and the left-right positioning of the visual shapes. This experimenter

was not involved with testing the infant. A second experimenter administered the tactual stimulus. He was not informed about the left-right positioning of the visual shapes. A third experimenter observed the infant's visual fixations through a 0.64-cm peephole in the centre of the rear wall of the testing chamber. He was unaware of both the tactual shape used and the left-right positioning of the visual shapes. Corneal reflections of the test objects were visible to this scorer, but the shapes of the objects were not resolvable. He scored the infant as fixating the left object when the left reflection was visible in either of the infant's pupils, and as fixating the right object when the right reflection was visible¹³. These fixations were recorded on a Rustack event recorder. Inter-observer agreement was high when assessed in both experiments (0.93, experiment 1; 0.98, experiment 2).

Thirty-two full-term infants ranging from 26 to 33 days old (mean 29.4 d) served as subjects in experiment 1. As infants of this age will not explore objects manually, the tactual stimuli were constructed by modifying pacifiers so that small, hard-rubber shapes could be mounted on them (Fig. 1). The matching shapes used for the visual test were constructed from dense Styrofoam and painted bright orange (diameter 6.4 cm). The experiment started with a 90-s tactual familiarisation period during which infants orally explored one of the tactual stimuli. This stimulus was then removed and the infant presented with both visual shapes for a 20-s visual test. Care was taken to ensure that the tactual stimulus was administered and removed without the infant seeing it. The shape used for tactual familiarisation, the left-right positioning of the visual objects, and the sex of the infants were counterbalanced. Thus, half the infants were tactually familiarised with the sphere and half with the sphere-with-nubs; half the infants in each of these groups were shown the familiar shape on the left and half on the right; half the infants within each of these subgroups were male and half female.

The results clearly demonstrate that infants under 1 month of age are capable of intermodal matching (Table 1). Of the 32 infants, 24 fixated the shape matching the tactual stimulus longer than the non-matching shape. These results were significantly different from chance ($P < 0.01$; binomial test). The mean per cent of total fixation time directed to the matching shape was 71.8%, as compared with the chance level of 50% ($t = 3.07$; $P < 0.01$). There were no significant differences due to sex of the infant, familiarisation object or method of feeding (breast or bottle), nor were there significant preferences for fixating the right versus left side or for fixating the sphere versus sphere-with-nubs.

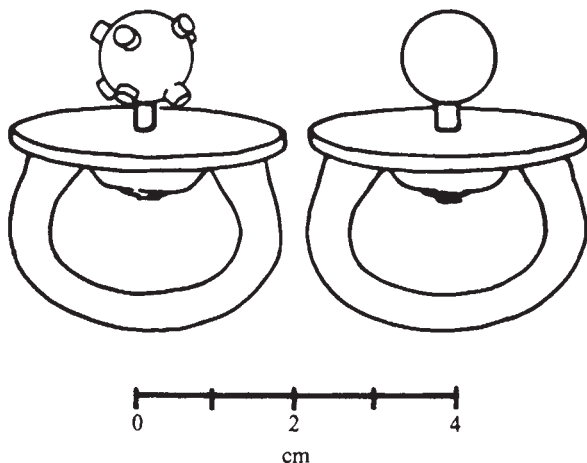


Fig. 1 The tactual objects. The tactual shapes were 1.2 cm in diameter. They were moulded from G.E. RTV-620A silicone rubber and fixed to a threaded nylon rod (6-32) that was bolted to the pacifier backing. The sphere-with-nubs was administered so that four of the nubs were orientated towards the base of the infant's mouth and four towards the roof. The nubs on the sphere were 2 mm high $\times$ 3 mm wide. The tactual stimuli were administered while the infant sat in an infant chair with his back to the visual objects. They were administered without the infant seeing them by having the experimenter hide them in his cupped hand while he was bringing the objects to and from the infant's mouth. The tactual familiarisation period started as soon as the infant began sucking on the tactual shape. Ten seconds before the end of the familiarisation period, the room lights were extinguished and the infant's seat was swivelled around so that he faced the visual objects. When the familiarisation period ended, the experimenter removed the tactual object, centred the infant's head midway between the visual objects, and only then switched on the light illuminating the visual objects. As soon as the infant made his first fixation to either object, the visual test period began, and the

experimenter removed his hands from the infant. The centres of the visual objects were 28 cm apart and 32 cm from the infant's eyes. They were displayed in a three-sided black cardboard chamber 71 cm high $\times$ 112 cm wide $\times$ 52 cm deep, and each suspended from the ceiling of this chamber by a thin black rod (diameter 0.64 cm). Illumination for the visual objects was provided by an incandescent bulb directly above and behind the infant's head, yielding a luminance of approximately $0.86 \log \text{cd m}^{-2}$ at the objects and $-0.14 \log \text{cd m}^{-2}$ at the background midway between the objects. The visual sphere-with-nubs was orientated so that four of the nubs directly faced the infant. Each nub on the visual object was 1.1 cm long and 1.2 cm in diameter and fashioned from a section of wooden doweling with the edge rounded.

Table 1 Numbers of infants who looked longer at the object matching one they had explored tactually, compared with those who looked longer at the non-matching object

	<i>n</i>	Looked longer at matching shape	Looked longer at non-matching shape	<i>P</i> (binomial test, two-tailed)
Expt 1	32	24	8	0.01
Expt 2	32	22	10	0.05

Studies using paired-comparison paradigms usually report a preference for the novel stimulus. The present findings are not incompatible with this, as there are several important procedural differences between the present experiments and those previously reported. For example, the present experiments (1) used infants younger than typically tested with the paired-comparison technique, (2) assessed tactual-visual rather than visual-visual matching, (3) used three-dimensional forms rather than two-dimensional patterns, (4) used a 90-s tactual familiarisation period. These differences may interact to influence the direction of infant visual preference.

A second experiment checked whether different experimenters using a different sample of infants could replicate the previous effects. A new group of 32 full-term infants 27–31 days old (mean 29.4 d) served as subjects. The procedure was identical to that used in experiment 1 and the effects were replicated (Table 1). Of the 32 infants, 22 fixated the matching shape longer than the non-matching one ($P = 0.05$). The mean per cent of total fixation time directed to the matching shape was 67.1% ($t = 2.14$; $P < 0.05$).

These experiments used a successive, intermodal matching task. The tactual object was no longer in the perceptual world at the time the infants were presented with the visual test. Positive results from such a task indicate that neonates can (1) tactually discriminate between the shapes presented, (2) visually discriminate between them, (3) store some representation of the tactually perceived shape, and (4) relate a subsequent visual perception to the stored representation of the tactually perceived shape. The last point has implications for our current theories of infancy.

A basic assumption of piagetian theory³ is that infants begin life with independent sense modalities that gradually become intercoordinated with development. Our findings, however, show that neonates are already able to detect tactual-visual correspondences, thereby demonstrating an impressive degree of intermodal unity. Thus, whatever develops in the first year of life, it is apparently not the *de novo* coordination of functionally independent sense modalities. A more general implication of the present research is that human neonates are not limited to processing bits of sense-specific information such as retinal images or tactual sensations. If neonates were restricted to registering such sensory elements they could not succeed on these intermodal matching tasks. Obviously, these initial experiments do not isolate the exact nature of the information perceived as invariant across the different modalities. However, they suggest that neonates are capable of using and storing surprisingly abstract information about objects in their world. This information must be abstract enough, at least, to allow recognition of objects across changes in size and modality of perception.

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Is there a 'consolidation' effect for monocular deprivation?

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During a time of high vulnerability early in a kitten's postnatal life, a few days of monocular vision can result in the control of most striate cortical neurones exclusively by the open eye^{1–5}. Although the general consequences of this procedure have been known for some time⁶, the mechanisms remain unknown. Because they develop rapidly^{3,4}, it has been suggested that the processes involved are similar to those of memory and learning. One aspect of this analogy is that memory is thought to be 'consolidated' from a weak to a stable form and that the process can be disrupted by the administration of drugs, electroconvulsive shock or physical trauma⁷. The connection with plasticity in the visual cortex has been suggested by studies in which kittens were first bilaterally deprived and then briefly exposed either monocularly or binocularly^{3,4,8}. The effects of exposure were reported to be greatest if there was a delay between exposure and physiological study. Although this has been referred to as a 'consolidation' effect^{3,4,5,8}, it was a delay process rather than a process of stabilisation that was investigated. Furthermore, even though the evidence for this effect is not compelling, it has been assumed that the consequences of monocular deprivation in kittens with prior normal rearing also are consolidated, that is, they become accentuated if the unilateral occlusion is followed by a period without vision⁵. We tested directly the notion that short-term monocular deprivation effects are more extensive if, after unilateral exposure, a period is imposed before physiological study during which kittens are kept in darkness. We report here that the consolidation idea is not supported by our results. On the contrary, the post-monocular period spent in darkness actually diminished the consequences of the deprivation, allowing some functional recovery of binocular connections.

Kittens were reared normally until postnatal day 29, when susceptibility to deprivation effects is very high¹. Vision through one eye was then blocked for 24 h using a large opaque contact lens. In one group of animals, study of cortical cells was begun immediately after the occlusion period. A second group was placed in a darkroom for 48 h before recording. Because 4-week-old kittens sleep a great deal, it was necessary to keep them awake during as much of the monocular exposure period as possible. All kittens were watched during most of their exposure sessions and they were periodically isolated from their mothers so that their attention could be maintained. The recording sessions were arranged so that cats were studied in pairs, one by each of us in separate laboratories. Findings were not compared until the studies were completed.

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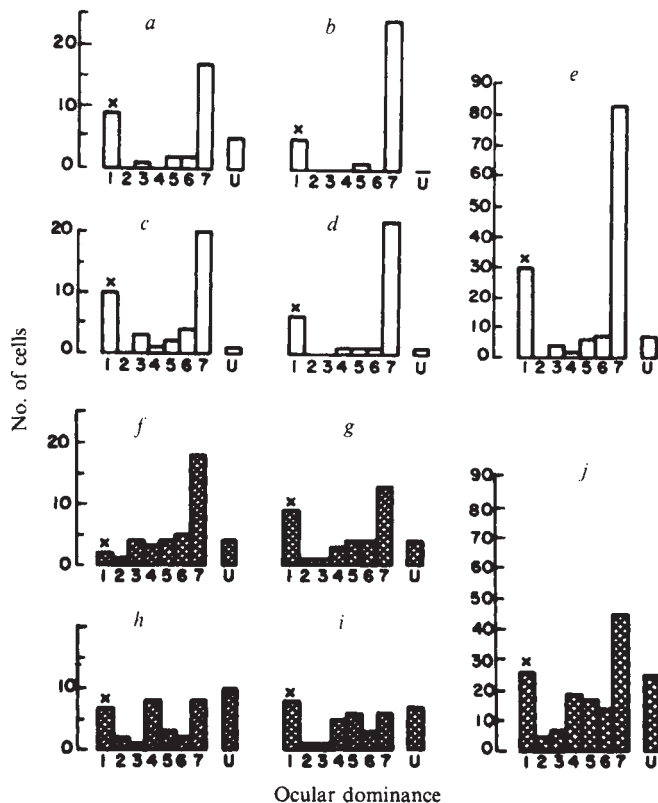


Fig. 1 Ocular dominance histograms are shown for experimental animals. The numbers on the abscissae represent subjectively determined classifications of the degree to which the two eyes influence a given cortical cell. Binocular neurones are designated by groups 2–6 and monocular cells by groups 1 and 7. Groups 1–3 (or 5–7) represent cells dominated by the eye contralateral (or ipsilateral, respectively) to the hemisphere from which electrode recordings were made. Visually unresponsive cells are indicated by the category 'U'. After 4 weeks of normal postnatal rearing, eight kittens were unilaterally occluded with large opaque contact lenses worn in their left eyes for 24 h. Half were studied physiologically immediately after occlusion (*a–d*) and the other half spent 48 h in darkness before recording (*f–i*). Summary histograms are shown for each group in *e* and *j*. × Indicates the occluded eye, which, in all cases, was contralateral to the hemisphere that contained the electrode.

Standard physiological recording techniques were used^{9,10}. Halothane was used while a vein was cannulated, and anaesthesia was continued with Brevital while a tracheal tube was positioned and an electrode placed. Animals were then paralysed and artificially respiration; CO₂, electroencephalogram, electrocardiogram and temperature were monitored. Spikes isolated from individual neurones by means of tungsten-in-glass microelectrodes were amplified and displayed. As a conservative procedure, electrodes were always placed in the hemisphere contralateral to the deprived eye because, in the normal cat, the contralateral eye tends to predominate⁹.

Receptive fields were determined for each responsive unit. Optimal stimuli were found for each eye and cells were classified into types using standard criteria^{1,2,9,10}. We paid particular attention to subjectively determined estimates of absolute response strength and to the relative strength of responses elicited through each eye (ocular dominance). Response strength classifications used were: unresponsive, weak, fair, good and vigorous. Relative strength was categorised into seven ocular dominance groups, as defined in Fig. 1 legend.

In normal cats and kittens, most cells in the visual cortex are binocular^{1,9}. Ten days of monocular occlusion in a 4-week-old kitten results in the open eye gaining control over nearly all cortical cells². One day of this procedure is sufficient to produce

a marked effect, as shown in Fig. 1. Ocular dominance histograms *a–d* are for kittens studied immediately after 24 h of monocular deprivation. A summary of these results is given in *e*. Two marked effects are evident. First, very few binocular cells were found; second, considerably fewer cells were controlled by the deprived eye in all cases (27% of the total are contralateral, that is, fall into groups 1–3). Most cells were highly responsive to optimal stimuli and, as shown in the histograms, very few were visually unresponsive. Data from the animals placed in darkness for 48 h following 24 h of monocular exposure are shown in Fig. 1*f–i*, with a summary in *j*. Once again, there is a clear reduction of binocularity, but it is noticeably less than in the first group (binocular cells constitute 14% in the first group *e* and 47% in the second *j*). The findings for the group which was studied after a delay also indicate a predominance of ipsilateral (groups 5–7) cells (64%). Although this is less than the proportion observed in the group recorded immediately after monocular exposure (73%), the difference is not statistically significant (χ^2 test). The summary histograms (Fig. 1*e, j*) show one other prominent difference between the two groups. Visually unresponsive cells comprise only 5% (7 cells) of the total in the first group *e*, but 16% (25 cells) in the second group. In addition to the higher proportion of unresponsive cells, the imposition of 48 h of darkness (second group) seemed, in general, to cause a noticeably reduced strength of visual responsiveness even when cells were driven through the eye that had not been deprived. More units habituated, had bursting responses, exhibited high spontaneous activity and seemed generally variable than in the other group. As a consequence, more cells were classified as weak or fair. Apparently, the relatively short period with no visual input was sufficient to cause some degenerative changes in cortical connections.

The results presented in Fig. 1 clearly show that the period spent in darkness following 24 h of monocular exposure does not produce a consolidation of the unilateral deprivation effect. On the contrary, a substantial increase occurs in the proportion of cells that can be driven through both eyes. However, the conclusion that a consolidation period does not accentuate the effects of monocular deprivation is limited to the time courses we chose for unilateral exposure and for the post-exposure period. It is possible that a consolidation effect occurs, but over a different time course. The loss of binocularity for the animals recorded after 24 h of unilateral deprivation was so marked that a greater effect would be difficult to discern.

We decided, therefore, to examine two other groups of kittens with a shortened time course of monocular exposure. In the first, animals were unilaterally occluded for 8 h on postnatal day 29 and then studied physiologically. The second group was similarly exposed monocularly, but recording was delayed for an additional 8 h during which the kittens were kept in darkness. Because of the very brief time, animals were exposed in a slowly rotating clear chamber to ensure that they were alert during most of the period. Once again, analysis of a sample of cortical cells showed that binocularity was severely reduced for both groups of cats, although not as much as for those shown in Fig. 1. In three cats recorded immediately after monocular deprivation, 36% of all cells were binocular, compared with 51% in three others recorded after 8 h of darkness. In addition, the ipsilateral eye (non-deprived) predominated in both cases, but controlled 70% of the sample from the first group and 58% from the second which had an 8-h interval between exposure and physiological study. As in the case of 24-h monocular deprivation, these findings do not support the notion of consolidation. The degree of binocularity is once again actually higher when a period intervenes between exposure and recording. Therefore, we conclude that for this condition also, there is no consolidation effect.

We have shown that periods as short as 8 h of monocular deprivation can cause functional disruption of binocular cortical connections. This finding is distinct from that of previous studies in which short periods of monocular exposure were preceded by binocular deprivation^{4,11}. In that case, the physiological effects

observed reflect a unilateral recovery of visual function and the mechanisms could be different from those of monocular deprivation. The fact that the effects we have found are so much greater than those previously reported⁵ may be due in part to the procedures we used to ensure maximum exposure during the monocular rearing periods. However, the major difference is probably attributable to use, in the previous study, of a post-monocular period of binocular deprivation.

In the conditions of our study, we conclude that the effects of short-term monocular deprivation do not become consolidated during a post-exposure period spent in darkness. The inactivation of pathways from a deprived eye must require some time, but it must be much shorter than the period of deprivation required to produce an effect detectable by our methods. We have not tested 'consolidation' in the original sense^{12,13}, because our study did not attempt to look at the issue of conversion of a deprivation effect from an unstable to a stable form. To do this, one might administer, for example, electroconvulsive shock or a protein synthesis inhibitor like puromycin, to kittens immediately after a period of monocular exposure to see if these agents interfered with the effects of the deprivation.

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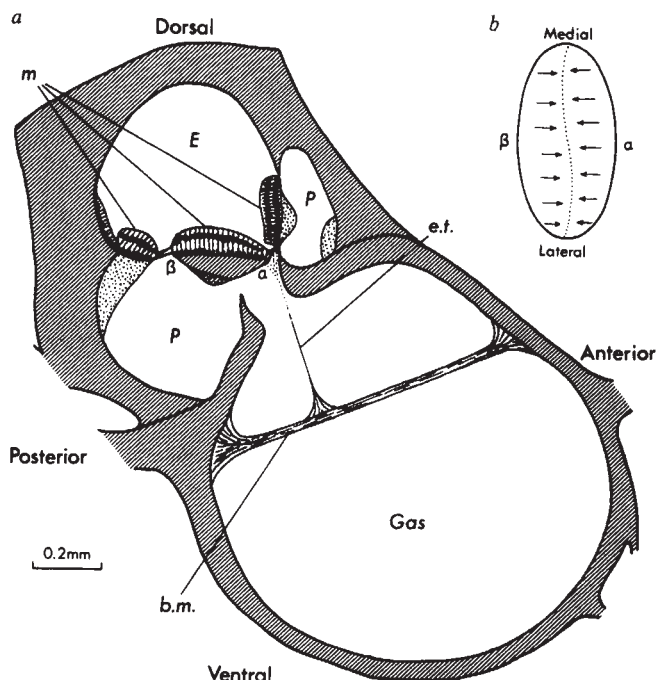


Fig. 1 a, Sagittal section through part of the auditory system of the sprat. Pressure vibrations cause vibrations of the bulla membrane (b.m.) which set up vibrations of the utricular maculae (m). The long axis of the middle macula is at right angles to the line $\alpha\beta$. The hatched areas are tough collagenous or calcified tissue. Nerves are stippled. The maculae are shown as cells but the 'hairs', cupulae and the utricular otolith are not shown. A thin elastic thread (e.t.) connects the bulla membrane to the base of the utricle (out of the plane of this drawing). It seems possible that this thread is associated with the detection of slow, large pressure changes. E is endolymph, P perilymph. Displacements of liquid are possible because the system communicates with the lateral line. b, Plan view from above of the middle macula of the utricle. It is divided into two areas in which the hair cells are orientated in opposite directions. The arrows point to the sides of the hair cells on which the kinocilia lie.

The analysis of sound by the sprat ear

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In the acoustico-lateralis systems of vertebrates the individual hair cells are usually polarised in their responses to displacements of the liquid in which they lie, and are often arranged in back-to-back pairs or groups with different polarities. A simple example to investigate, mechanically as well as electrically, is the utricle of the sprat (*Clupea sprattus* L.). The acoustico-lateralis system of the sprat and other clupeids has two partly gas-filled bony bullae which transform pressure changes into liquid displacements capable of stimulating the sense organs of the ear and lateral line¹⁻³. With its related structures the utricle is a very sensitive sound pressure detector which has one population of receptors that respond to the compressions and another that respond to the decompressions of a sound wave. We now give additional evidence that this type of organisation is unlike that of the mammalian cochlea in being specialised more for the detection of phase/time relationships than for frequency analysis.

Popper and Platt⁴ have described the distribution and orientation of hair cells in the three maculae of the herring utricle⁵. In each macula the hair cells have their kinocilia on the side closest to a line running down the middle of the long axis. One of us (J.A.B.G.), with A. C. G. Best, has independently made similar observations on the sprat utricle. This is like that of the

herring except that the hair cells of the posterior macula are orientated along, rather than across, its length. The anterior and middle maculae are suspended along their long edges (Fig. 1). Each sprat utricle has about 13,000 hair cells, about 60% on the middle macula and slightly more than 20% on the anterior macula.

As an electrode is introduced into the utricular endolymph a positive d.c. potential step of 10-14 mV is observed. On stimulation with sinusoidal pressure waves microphonic potentials of double frequency^{6,7} are recorded. The potentials consist of two sets of negative peaks, one associated with compressions, the other with decompressions (Fig. 2a). Because in fish, hair cells are preferentially excited when their ciliary processes are displaced towards their kinocilia⁸, it seems almost certain that the two sets of peaks correspond to the excitation of the two major populations of oppositely orientated hair cells in the two larger maculae. These responses do not change significantly in shape over a frequency band of at least 5-880 Hz. The two sets of peaks are similar in the way they change when the electrode is moved in and out of the endolymph, in the way they decline with anoxia and in the time intervals between the peaks of each set and the corresponding compressions or decompressions. The recorded responses to a pressure of 1 N m⁻² are of a few tens of microvolts and attain about half a millivolt at pressures around 100 N m⁻². We shall call the responses to compression c-responses (from c-receptors) and the responses to decompression d-responses (from d-receptors). The pressure required to give a particular response of either group changed very little between about 40 and 700 Hz. Below about 20 Hz sensitivity declined sharply.

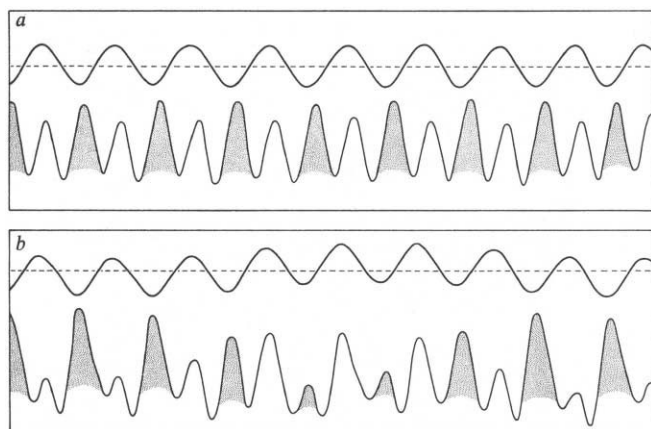


Fig. 2 *a*, Upper trace: stimulus, a sinusoidal pressure wave of 200 Hz; mean pressure shown by dashed line, compressions upwards; peak to peak pressure 12.5 N m^{-2} . Lower trace: potentials in the endolymph of the utricle; *d*-responses are stippled. *b*, Like *a* but the stimulus pressure obtained by mixing frequencies of 200 Hz (as in *a*) and 30 Hz.

Two frequencies of pressure stimulation can be combined (see ref. 7) as, for example, in Fig. 2*b*, where 200 and 30 Hz waves are mixed. The *d*-responses to the 200-Hz component (stippled) are larger during the decompression phase of the slower wave and smaller during the compression phase. The *c*-responses behave in the reverse way. In Fig. 2 the envelopes enclosing both the *c*- and *d*-responses correspond to the lower frequency. The two frequencies and their phase relationship are much clearer in the responses of the receptors than in the pressure record. The responses of the two different receptor populations result from pressure excursions either above or below the mean pressure and their timing can be predicted from the form of the pressure wave. The sizes of the responses increase with the extent of the compression or decompression, but this relationship is not the same as that of the *d*-response to simple sine wave stimulation, for the slope of the log response/log pressure curve is much steeper.

The phenomena described above also produce potential patterns which are very sensitive to phase between harmonics. Figure 3 shows utricular responses to changes of phase between a pressure wave of 62.5 Hz and its second harmonic.

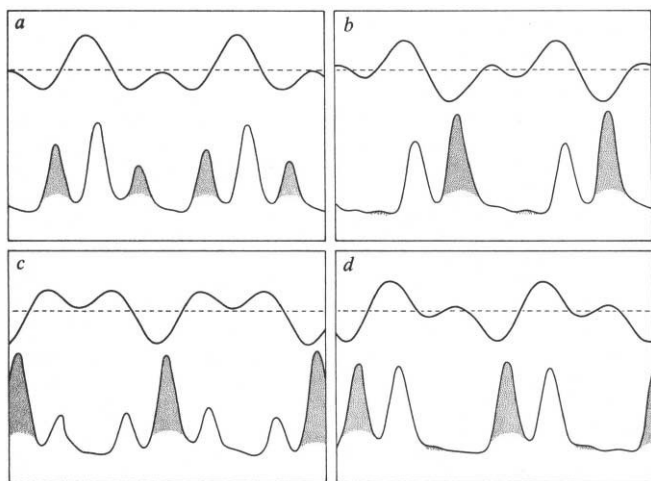


Fig. 3 To show that phase changes greatly affect the pattern of the responses given by the utricle. *a*, Upper trace: stimulus pressures obtained by mixing frequencies of 125 Hz and 62.5 Hz of approximately equal amplitudes ($\sim 15 \text{ N m}^{-2}$); mean pressure shown by dashed line, compression upwards. Lower trace: potentials in the endolymph of the utricle; the *d*-responses are stippled. *b*, *c*, *d*, Like *a* but with the higher frequency delayed by 2, 4 and 6 ms, respectively (relative delays in phase 90° , 180° and 270°).

These results give information on the representation of amplitude and timing in the activity of the utricle. As a considerable proportion of the 13,000 cells is likely to be available, it seems probable that amplitude can be coded accurately and quickly by the number of active units in the population of nerve fibres. The intervals between compressions or decompressions or between a compression and a decompression are clearly represented even in a complex wave by the activities of the two populations of receptors. The system should allow interactions which diminish noise and blurring. The striking changes in the relative amplitudes of the *c*- and *d*-responses which immediately follow changes in the pressure wave strongly suggest that the system is organised to facilitate the detection of time/phase differences and provide rapid answers to rapid events, that is, to noises or transients given, for example, by a sharp change of direction of a neighbouring fish⁹. It also provides a 'standard' based on pressure against which the activity of the various neuromasts of the lateral line can be compared, thus enabling estimates of distance and direction to be made^{3,10}.

As so much is known about hearing in mammals, it is interesting to contrast the behaviour of the clupeid utricle with that of the mammalian cochlea. Microphonics recorded from the latter are very different from those described above; there is no frequency doubling and the response to a sinusoidal stimulus is an almost perfectly sinusoidal wave of the same frequency (see for example, ref. 11).

Helmholtz and others have shown that, with trivial exceptions, man is insensitive to phase relationships of tones^{12,13}. Results like those of Figs 2 and 3 support the view that this may not be true for animals in which the receptors are organised in the same general way as the sprat utricle.

J.A.B.G. is a member of the external scientific staff of the MRC.

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Met- and Leu-enkephalin immunoreactivity in separate neurones

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A pair of pentapeptides, Met- and Leu-enkephalin were recently isolated from brain tissue^{1,2}. The two peptides seem to represent endogenous opiate receptor ligands and have by immunocytochemical and radioimmunoassay studies been shown to occur in an extensive system of cerebral and peripheral nerves³⁻⁸. The relative proportions between Met- and Leu-enkephalin varies between different brain regions and also between different species, suggesting the existence of separate populations of Met- and Leu-enkephalin nerves⁹⁻¹². Until now, however, immunocytochemistry has given no support for this notion. We report here evidence of separate populations of Met- and Leu-enkephalin nerves.

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Table 1 Properties of enkephalin antisera determined by radioimmunoassay

Serum	Dilution	IC ₅₀ (pmol of peptide)		
		Met-enk	Leu-enk	β -endorphin
anti-Met-enk M12	1:250	40	4,500	>25,000
anti-Met-enk M14	1:250	25	2,000	>25,000
anti-Leu-enk L2	1:500	500	3	>25,000

Radioimmunoassays were performed as previously described²¹, using a dilution of serum which bound 30–40% of ³H-enkephalin in the absence of unlabelled peptide. Results of competitive binding experiments are expressed as the IC₅₀ values of the amounts of peptides required to displace 50% of bound ³H-enkephalin in the radioimmunoassay. The incubation volume was 0.25 ml. The data were obtained from typical experiments performed in triplicate and repeated at least twice.

Met- and Leu-enkephalin differ only in that their COOH-terminal residue is either methionine or leucine. Furthermore, Met-enkephalin is identical to the NH₂-terminal pentapeptide of the long-chain opioid peptide, β -endorphin. So far, however, there is no evidence for a biosynthetic relationship between these two peptides. Furthermore, immunocytochemistry has demonstrated that enkephalin-immunoreactive nerves show a distribution distinct from that of nerves containing β -endorphin, β -lipotropin (β -LPH) and adrenocorticotrophic hormone (ACTH)^{6,13,14}. The similarities in amino acid sequences may, however, cause significant problems of cross-reactivity between different opioid peptides. In several previous studies, radioimmunoassay data have been obtained indicating only small degrees of cross-reactivity between the two enkephalins^{11,12}. Such data cannot, however, be taken as proof for immunocytochemical specificity^{15,16}. Thus, of numerous reports on enkephalin immunocytochemistry^{3–8,17} to our knowledge, only one, dealing with Met-enkephalin localisation⁵, seems to have fully documented lack of cross-reactivity.

We have examined a number of antisera to Met- and Leu-enkephalin in immunocytochemical models and have obtained evidence for considerable cross-reactivity. With selective absorptions as well as by oxidative destruction of the methionine residue of Met-enkephalin we have been able to develop methods, selectively demonstrating either Met- or Leu-enkephalin. When applied to tissue sections, these selective techniques reveal that the two enkephalins are localised in two distinct systems of nerves.

Three antisera were used (Table 1). In radioimmunoassay both Met-enkephalin antisera, M12 and M14, displayed approximately 100-fold greater selectivity for Met-enkephalin than Leu-enkephalin. The Leu-enkephalin antiserum L2 had over 150 times more affinity for Leu-enkephalin than Met-enkephalin. None of the three antisera exhibited any cross-reactivity with concentrations of β -endorphin as high as 10⁵ pmol ml⁻¹.

Cytochemical models consisted of Sepharose 4B beads, to which was coupled either synthetic Met-enkephalin, Leu-enkephalin or β -endorphin. Treated beads were attached to glass slides by the technique of Streefkerk *et al.*¹⁸ and subjected to immunocytochemical staining (peroxidase–antiperoxidase, PAP procedure). L2, M12 and M14 antisera were all found to react with beads to which Met- and Leu-enkephalin was coupled, whereas, at all concentrations tested (1:200–1:40,000), they failed to react with the β -endorphin beads. In accord with this observation, pre-adsorptions of the three antisera with β -, α -, and γ -endorphin, ACTH or other unrelated peptides (see Fig. 1) failed to affect staining of the Met- and Leu-enkephalin beads. Pre-adsorption of all three antisera with Met-enkephalin (2–100 μ g ml⁻¹) resulted in complete inactivation of staining of both Met- and Leu-enkephalin beads. In contrast, pre-adsorption of antisera M12 and M14 with

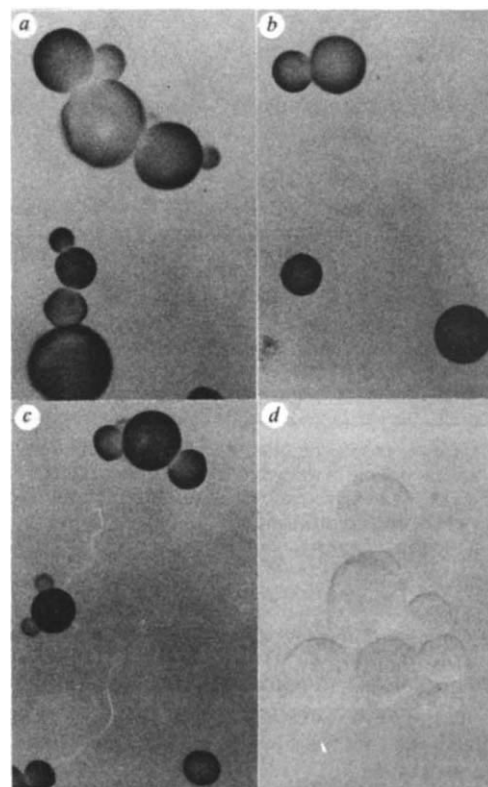


Fig. 1 Sepharose beads to which Leu-enkephalin (a, b) or Met-enkephalin (c, d) has been coupled. The beads shown in a and c have been reacted with Leu-enkephalin antiserum (L2; PAP technique) without pretreatment, whereas the beads in b and d have been pre-oxidised with acidic permanganate before staining with antiserum L2. Note that this antiserum reacts as readily with Met- as with Leu-enkephalin (a, c) and that oxidation with acidic permanganate eliminates staining of Met- (d) but not of Leu-enkephalin (b). Thus, following this type of pre-oxidation, antiserum L2 is fully specific for Leu-enkephalin. Similar model staining experiments confirmed that Leu-enkephalin-adsorbed antisera M12 and M14 were fully specific for Met-enkephalin. Cytochemical models were prepared by coupling synthetic Met-enkephalin, Leu-enkephalin or β -endorphin (Bioproducts Peptide and Peninsula Labs) to CnBr-activated Sepharose 4B beads (Pharmacia). In general, 1 mg of peptide was coupled to 2 ml swollen beads according to the manufacturers manual. Prepared beads were mounted as monolayers on glass slides according to Streefkerk *et al.*¹⁸ and subjected to various treatments before immunocytochemical staining. Such treatments included exposure to solutions used for preparing the tissue material (compare with Fig. 2) and excluded deleterious influences of these procedures on the Met- or Leu-enkephalin immunoreactivity. In addition, the beads were treated with solutions of potassium permanganate in dilute sulphuric acid. The best formula agreed with that for antibody elution suggested by Tramu *et al.*¹⁹, and consisted of 0.15 M KMnO₄ and 0.02 M H₂SO₄. Treatment of beads (or cryostat sections) with this solution for 2 min was sufficient to completely block Met-enkephalin immunoreactivity, whereas even a 10-min exposure left Leu-enkephalin immunoreactivity unaffected (as tested by antiserum L2). Oxidised and non-oxidised beads were allowed to react with either unpretreated, Leu-enkephalin-pretreated or Met-enkephalin-pretreated antisera to Met-enkephalin (M12, M14) or Leu-enkephalin (L2). Antisera were produced in rabbits by immunisation with the enkephalins coupled by carbodiimide and glutaraldehyde to keyhole limpet haemocyanin, as previously described²¹. The site of antigen–antibody reaction was revealed by either PAP or immunofluorescence²². Controls for immunocytochemical specificity were made by pretreating antisera with varying amounts of Leu- or Met-enkephalin (2–100 μ g ml⁻¹ diluted antiserum) as well as with excess amounts of α -, β - and γ -endorphins, ACTH (Ferring) and synthetic human gastrin I (ICI). Of these peptides, only the enkephalins affected staining and additional controls including conventional staining controls²², gave negative results.

Leu-enkephalin ($5\text{--}100\text{ }\mu\text{g ml}^{-1}$) failed to abolish staining of the Met-enkephalin beads, whereas it abolished staining of the Leu-enkephalin beads. On the basis of these results we conclude that the Met-enkephalin antisera M12 and M14 contain two antibody populations: one specific for Met-enkephalin and one reacting with both Met- and Leu-enkephalin. The latter antibody population could be inactivated by pretreating the antisera with as little as $5\text{ }\mu\text{g}$ of Leu-enkephalin, but in all subsequent studies this antiserum was used routinely with $100\text{ }\mu\text{g}$ Leu-enkephalin added per ml of a $1:1,600$ dilution. In these conditions, both antiserum M12 and M14 are fully specific for Met-enkephalin.

We noted that even mild oxidative treatment (by acidic permanganate or cyanogen bromide) completely abolished immunoreactivity of the Met-enkephalin beads, whereas that of Leu-enkephalin beads was left unaffected. This effect can be explained by destruction of the methionine residue of Met-enkephalin and has previously been exploited in bioactivity studies^{9,10}. Thus, following such oxidative treatment our cross-reactive antiserum L2 produced strong staining of Leu-enkephalin beads but left Met-enkephalin beads unstained (Fig. 1).

On the basis of such model experiments we examined the tissue distribution of Met-enkephalin by using Leu-enkephalin-adsorbed antiserum M14 or M12, and of Leu-enkephalin by treating tissue sections with oxidising agents (usually acidic permanganate solutions), before staining with Leu-enkephalin antiserum L2. Immunocytochemical specificity was proved by adsorbing antisera against the enkephalins, endorphins, ACTH and other peptides (compare Fig. 1). As precursors to the enkephalins have not yet been identified it is impossible to assess whether our antisera would also react with such molecules. Note, however, that although β -endorphin contains the entire sequence of Met-enkephalin, none of the antisera used reacts with this long-chain opioid peptide. Even if our enkephalin antisera did react with enkephalin precursors, however, our results make it reasonable to assume that oxidation plus staining with antiserum L2 demonstrates only Leu-enkephalin-related molecules and that Leu-enkephalin-pre-adsorbed antiserum M12 and M14 react only with Met-enkephalin-related molecules.

Both techniques demonstrated numerous immunoreactive nerves in both the gastrointestinal tract of cats and guinea pigs and in brains of guinea pigs. In most regions studied, the number of Met-enkephalin nerves exceeded that of Leu-enkephalin nerves. The two types of nerves showed roughly the same distribution, which agreed with previous data on the distribution of enkephalin immunoreactivity in brain and gut^{3-8,17}. In the cat gastrointestinal tract, occasional ganglia of the myenteric plexus seemed to contain predominantly Leu- or Met-enkephalin nerves (Fig. 2). Most areas examined, including the myenteric plexus showed, however, great parallelism in the distribution of the two types of nerves. To prove that the two peptides did occur in distinct populations of nerves, double-staining immunocytochemistry was carried out. Met-enkephalin immunoreactivity was first demonstrated by Leu-enkephalin-pre-adsorbed antiserum M12 or M14 with the peroxidase-antiperoxidase (PAP) procedure. Following demonstration of peroxidase activity with 3,3'-diaminobenzidine, which produced a brown reaction product, antibodies were eluted from the sections by the method of Tramu *et al.*¹⁹. This technique uses an acidic permanganate solution, which also destroys Met-enkephalin immunoreactivity. Following successful elution and simultaneous destruction of Met-enkephalin immunoreactivity, the sections were restained with antiserum L2, now able to react only with Leu-enkephalin present in the sections. The PAP technique was used again, but peroxidase activity was now demonstrated with 4-Cl-1-naphthol, yielding a blue reaction product¹⁹. Thus Met-enkephalin-containing nerves are stained brown and Leu-enkephalin-containing nerves are stained blue.

Alternatively, the site of reaction between the L2 antiserum and the tissue was shown by indirect immunofluorescence. In this case, brown-stained Met-enkephalin and immuno-

fluorescent Leu-enkephalin nerves were visualised (Fig. 3). The success of the antibody elution was verified by the fact that the Met-enkephalin nerves did not restain in the second step of the double-staining procedures as well as by controls involving use of antisera pre-adsorbed with either Met- or Leu-enkephalin in both steps of the procedures and, finally, also by the substitution of the enkephalin antisera by unrelated immune sera. All these controls confirmed the specificity of the immunolocalisations and also documented the completeness of the antibody elution technique as well as its ability to destroy Met-enkephalin reactivity to antiserum L2. The double staining techniques were successful in demonstrating distinct populations of Met- and Leu-enkephalin nerves in the nucleus caudatus-putamen and globus pallidus of the guinea pig brain and in the gastrointestinal tract. Interestingly, both types of nerves showed a very similar distribution, with Leu-enkephalin nerves being about one-quarter to one-fifth as numerous as Met-enkephalin fibres. A full description of the extent and distribution of the two types of nerves will be published elsewhere.

Our data demonstrate that Leu- and Met-enkephalin occur in two distinct populations of nerves. The occurrence of two distinct types of enkephalin nerves is of considerable interest, partly because both types seem to show at least partially overlapping distributions. This distribution also agrees with that of

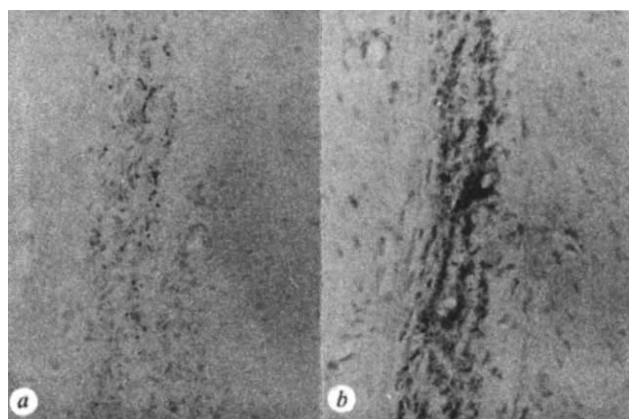


Fig. 2 Adjacent sections showing Auerbach's plexus of cat ileum. *a*, This section has been stained with Leu-enkephalin-adsorbed antiserum M14 (PAP technique), whereas *b* has been pre-oxidised with acidic permanganate and then stained with antiserum L2 (compare with Fig. 1). Thus, in *a* Met-enkephalin immunoreactive nerves and in *b* Leu-enkephalin immunoreactive nerves are shown. Note that in this particular ganglion the Leu-enkephalin nerves are more numerous than the Met-enkephalin nerves and that also a Leu-enkephalin immunoreactive cell body can be seen. Similar experiments, involving staining of the same section for the two types of immunoreactivity in contrasting colours (compare with Fig. 3), showed that the Leu- and Met-enkephalin immunoreactivity resided in separate nerve populations. Guinea pigs ($n = 10$) and cats ($n = 5$) were anaesthetised with diethyl ether or mebumal chloralose, respectively, and perfused via the heart with cold (4°C) saline followed by a 4% paraformaldehyde solution in 0.1 M sodium phosphate buffer pH 7.3. Specimens from the gastrointestinal tract of both species as well as frontal brain slices from guinea pigs were postfixed in the paraformaldehyde solution for 15–30 min, soaked in 20% sucrose solution in the phosphate buffer overnight and frozen on cryostat chucks in melting Freon-22. Cryostat sections, cut at $3\text{--}10\text{ }\mu\text{m}$, were subjected to immunocytochemical staining for Met- and Leu-enkephalin immunoreactivity, as described in Fig. 1 legend. Controls were also identical. Occasionally, sections stained for Met-enkephalin immunoreactivity were submitted to antibody elution according to Tramu *et al.*¹⁹ and poststained with a cross-reacting Leu-enkephalin antiserum (L2). This elution technique is identical with that used for destroying Met-enkephalin immunoreactivity. Met- and Leu-enkephalin nerves could be demonstrated in contrasting colours, using the PAP technique. The efficiency of antibody elution was shown by the fact that separate nerves were stained, and by the controls, suggested by Tramu *et al.*¹⁹.

brain opiate receptors⁴. As previously pointed out, nerves containing other putative ligands for opiate receptors, like β -endorphin and ACTH, show only a partially overlapping distribution with enkephalin nerves^{6,13,14}. These data should be viewed in the light of the previously suggested existence of separate types of opiate receptors²⁰ and should initiate studies of differential release of the two endogenous opiate ligands.

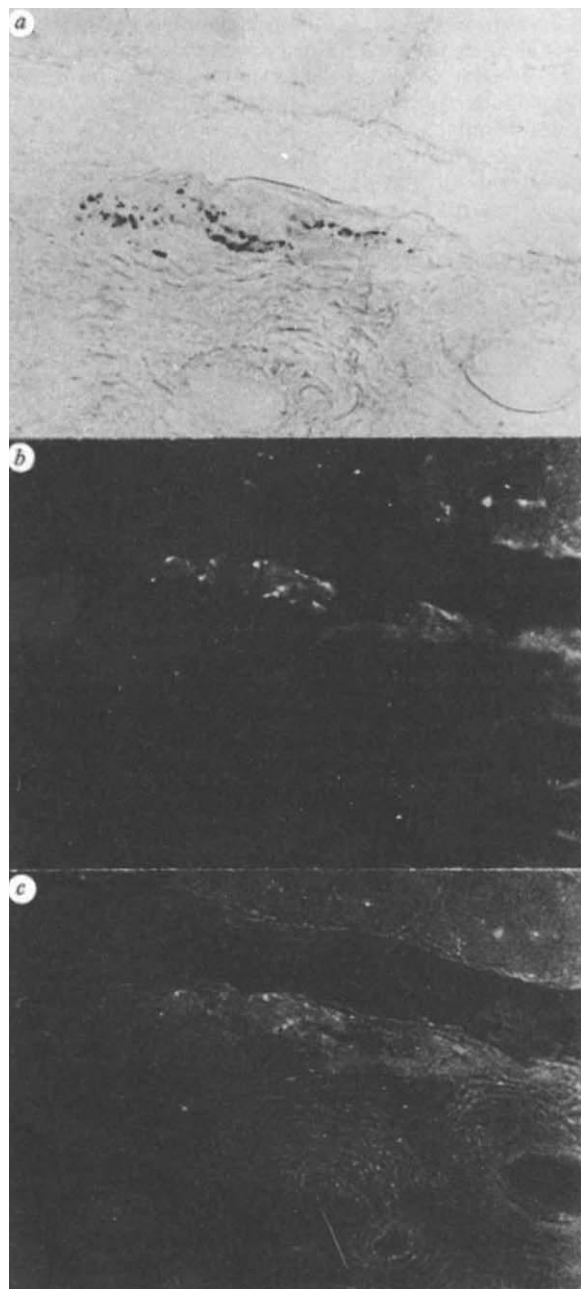


Fig. 3 Section of submucous (Meissner's) ganglion of cat small intestine. The section was first stained with Leu-enkephalin-adsorbed antiserum M14 using the PAP procedure (top) resulting in dark-stained Met-enkephalin nerves. Subsequently, antibodies were eluted and Met-enkephalin immunoreactivity destroyed by acidic permanganate treatment, whereafter the section was restained with antiserum L2, now able to show only Leu-enkephalin immunoreactivity (compare with text and Fig. 2). The site of reaction with the L2 antiserum was revealed by indirect immunofluorescence, using fluorescein isothiocyanate-labelled goat antirabbit IgG (centre). Bottom, the same section is viewed in combined blue light (490 nm) and in ordinary light, showing that the black-stained Met-enkephalin nerves are distinct from the immunofluorescent Leu-enkephalin nerves. $\times 370$.

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A novel analgesic dipeptide from bovine brain is a possible Met-enkephalin releaser

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It is generally accepted that morphine exerts its analgesic effect by binding to specific opiate receptors in the brain and spinal cord¹. Since Hughes *et al.*² isolated and identified two endogenous pentapeptides, Met- and Leu-enkephalin, from the brain and found that they acted as agonists at opiate receptors, α -, β - and γ -endorphins, larger peptides than enkephalins and having morphine-like activity, have been identified in either the brain or pituitary of various species¹. Several studies have demonstrated that enkephalins possess analgesic properties and that they are distributed in the pain-mediated pathways in the central nervous system. These findings suggest that enkephalins are important neurotransmitters or neuromodulators regulating pain transmission. We now report the isolation of a novel substance which has a Met-enkephalin releasing action. Our findings suggest the possibility of a regulating mechanism for the release of endogenous opioid peptides, especially Met-enkephalin.

During purification, bovine brain extracts were injected intracisternally into unanaesthetised mice, using a J-shaped needle³, and analgesia was evaluated by the tail-pinch test using an artery clip (2 mm wide) with a constant pressure of 200 g (ref. 4). All the materials tested were dissolved in distilled water and administered into the cisterna magna, in a volume of 10 μ l. Eight mice were used for each experiment. The specificity of morphine-like activity was confirmed using the narcotic antagonist naloxone.

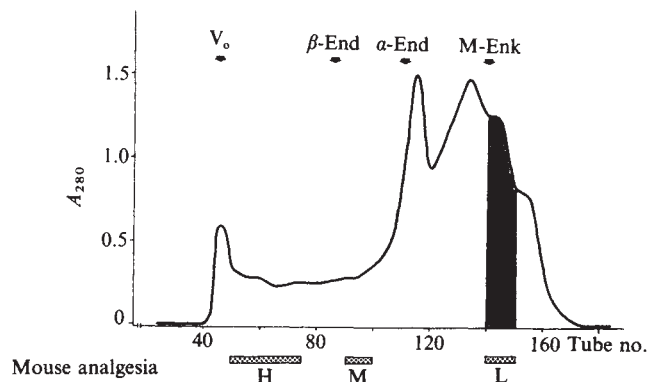


Fig. 1 Gel filtration chromatography of 3 g of 0.5 M acetic acid extract on a Sephadex G-50 column (90×5 cm). Elution was carried out with 0.5 M acetic acid, the rate of flow was 1 ml min⁻¹, and 15-ml fractions were collected. The elution pattern was monitored by UV absorbance at 280 nm. The analgesic activities were found in three fractions, H, M and L, indicated by the horizontal bars. The approximate elution positions of some endorphins are indicated by the arrows. V₀, void volume; β -End, β -endorphin; α -End, α -endorphin; M-Enk, Met-enkephalin.

Isolation of the substance was as follows. Fresh frozen bovine brains minus cerebella were crushed and homogenised in 5 volumes of acetone with a Polytron homogeniser at -10 °C. The homogenate was filtered and the acetone powder obtained was stirred for 12 h with 10 volumes of 0.5 M acetic acid at 4 °C. After centrifugation, the supernatant was lyophilised, redissolved in a small volume of 0.5 M acetic acid and applied to a Sephadex G-50 column. As shown in Fig. 1, analgesic activities measured as described above were found in three fractions, designated H (molecular weight ~10,000), M (MW ~3,000) and L (MW ~1,000). As the naloxone-induced reversibility of fraction L was more clear-cut than that of the other two fractions, this fraction was run in a Dowex 50W-X2 column to separate the peptides further. The column was eluted with a linear pH gradient of 0.2 M pyridine-acetate buffer, pH 3.1, and 1 M pyridine-acetate buffer, pH 7.0, and successively with 0.01 M NH₄OH, 1 M NH₄OH and 2 M NaOH. Analgesic activities were found in three fractions, L1, L2 and L3. The peptides of fraction L3 were found to be basic, as they could be

eluted with 1 M NH₄OH. Fraction L3, which consisted of three peaks, could be further broken down into three fractions, L3a, L3b and L3c (see Fig. 2). In these five fractions (L1, L2, L3a-c), the most potent analgesic activity was found in fraction L3b, which showed a 38-fold enrichment in analgesic activity compared with the original L fraction. Fraction L3b was then applied to a BioGel P-2 column (Fig. 3), and the L3b' fraction obtained showed an analgesic activity 1.5-fold greater than that of fraction L3b. The analgesic effect of intracisternally injected L3b' peptide (12 μ g per mouse) was maximal (about 55%) within 5 min, terminated 30 min after administration and was completely suppressed by pretreatment with naloxone (0.5 mg per kg, subcutaneously (s.c.)). The elution position of fraction L3b' did not correspond with the positions of α -endorphin or glycine, and showed that this substance had a relatively small MW. With high-voltage paper electrophoresis (HVPE) at 33 V cm⁻¹ for 90 min at pH 3.6 (pyridine/acetic acid/H₂O; 1:10:289) fraction L3b' showed a single spot with ninhydrin reagent. The relative mobility value of peptide L3b' was 0.75 using arginine as a standard, and its amino acid composition, after hydrolysis with 6 M HCl at 110 °C for 18 h, was tyrosine 0.90 and arginine 1.00. Tryptophan was not detected with Ehrlich reagent on TLC of peptide L3b'. Moreover, dansylation and HCl hydrolysis of the peptide resulted in a single spot on the polyamine sheet corresponding to *bis*-tyrosine, and thus indicating that the single N-terminal amino acid was indeed tyrosine. In view of these results and the elution position on the BioGel P-2 column, tyrosyl-arginine is proposed as the amino acid sequence of peptide L3b'.

In comparative studies, synthetic dipeptide Tyr-Arg and the endogenous peptide L3b' had an identical R_F value (R_F 0.18) on TLC (*n*-butanol/pyridine/acetic acid/H₂O; 4:1:1:2), showed only a single spot on co-chromatography, and had a corresponding retention time on HPLC. Similar results were obtained in the HVPE in the above conditions (R_F 0.75 using arginine as a standard). Finally, intracisternally administered synthetic Tyr-Arg and endogenous peptide L3b' had equipotent analgesic effects. These findings confirm that the endogenous analgesic compound, peptide L-3b', is a dipeptide of amino acid sequence Tyr-Arg. In preliminary experiments, Tyr-Arg could also be isolated from the 0.4 M perchloric acid extract of the mouse brain, by which the proteolytic enzymes are denatured. It is interesting that the Tyr-Arg sequence is present in some hypothalamic hormones—residues 74–75 and 79–80 in human thyroid stimulating hormone, residues 78–79 in human luteinising hormone, and residues 5–6 in human, but not bovine,

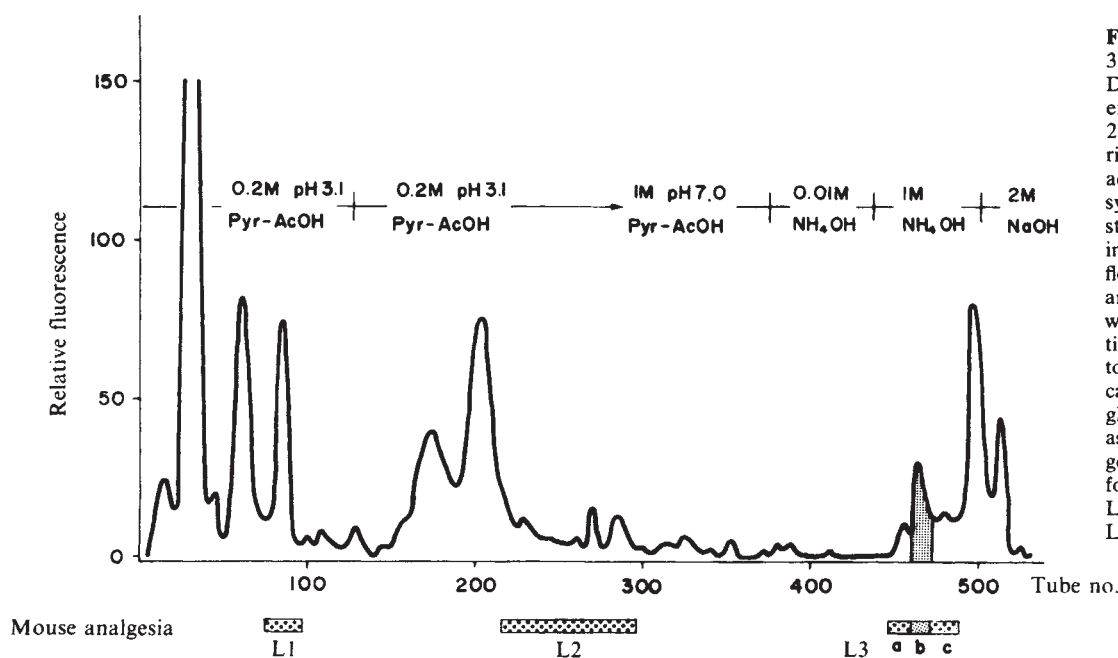


Fig. 2 Fractionation of 3 g of fraction L on a Dowex 50W-X2 cation exchange column (80×2 cm). Elution was carried out with a pyridine-acetate linear gradient system and using a stepwise system, as indicated. The rate of flow was 0.3 ml min⁻¹, and 15-ml fractions were collected. The elution pattern was monitored by the fluorescence method with glycine (10⁻⁸ M = 100) as a standard. The analgesic activities were found in five fractions; L1, L2, L3a, L3b and L3c, as indicated by the horizontal bars.

β -melanotropin. It remains to be determined whether these hypothalamic peptides are precursors of the newly identified dipeptide.

When injected intracisternally, the analgesic effect of synthetic Tyr-Arg was dose dependent and was completely abolished by pretreatment with naloxone (0.1 mg per kg, s.c.). The ED₅₀ value tested by the tail-pinch method was 34.7 nmol per mouse (11.7 μ g per mouse) with 95% confidence limits of 22.0–54.9 nmol. Although the analgesic potency of Tyr-Arg was about 60 times less than that of morphine (ED₅₀ 0.61 nmol per mouse), it was about 4.2 times higher than that of Met-enkephalin (ED₅₀ 146 nmol per mouse). In addition, the analgesic effect of Tyr-Arg lasted longer (for 30 min at ED₅₀ dose) than that of Met-enkephalin (disappeared within 15 min at ED₅₀ dose). Tyr-Arg-induced analgesia was also demonstrated by the hot-plate method of Loh *et al.*⁵, giving an ED₅₀ of 15.7 nmol per mouse. Furthermore, Tyr-Arg showed analgesic activity in the rat when given into the nucleus reticularis gigantocellularis, an area of the brain which is highly sensitive to morphine analgesia⁶.

Nevertheless, Tyr-Arg was only weakly inhibitory towards electrically induced contraction of longitudinal muscle of the guinea pig ileum (18.8% inhibition at 360 μ M), suggesting that this compound does not interact with opiate receptors. We therefore studied the binding affinity of Tyr-Arg to specific opiate receptors. The binding assay, using the rat brain membrane preparation, was as described by Simantov *et al.*⁷. Tyr-Arg was only weakly inhibitory towards ³H-dihydromorphine (24.9% inhibition at 100 μ M) and ³H-naloxone (22.7% inhibition at 100 μ M), indicating that the dipeptide does not bind with the specific opiate receptors. This strongly suggests that the naloxone-reversible analgesic effect of Tyr-Arg is mediated by enkephalins or β -endorphin. Therefore, Tyr-Arg acts either as an enkephalin- or β -endorphin-releasing factor or as an inhibitor of the degradation enzymes of enkephalins, such as aminopeptidase and enkephalinase⁸.

Met-enkephalin release was studied using the method described by Henderson *et al.*⁹. Its release from guinea pig striatal slices (four striata from two animals weighing ~600 mg) was detected by an opiate receptor binding assay after purification of the Met-enkephalin using HPLC as described by Meek and Bohan¹⁰. The basal release of Met-enkephalin in the superfusate (15-min collection period) was 16–17 ng. During 15 min of high-potassium ion (50 mM) stimulation, Met-enkephalin release increased to levels about four times higher than in the preceding spontaneous release samples. After 15 min exposure to a Tyr-Arg-containing medium (10⁻⁵ or 10⁻⁶ M) the output of Met-enkephalin increased to 2.3 times that of the preceding basal release (see Fig. 4).

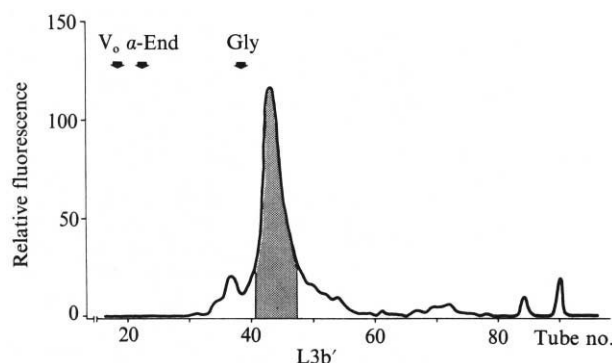


Fig. 3 Gel filtration chromatography of 8 mg of fraction L3b on a BioGel P-2 column (100 × 1 cm). Elution was carried out with 1 M acetic acid, the rate of flow was 0.2 ml min⁻¹, and 1.5-ml fractions were collected. The elution pattern was monitored by the fluorescence method with glycine (10⁻⁸ M = 100) as a standard. The approximate elution positions of α -endorphin (α -End) and glycine (Gly) are indicated by the arrows. V₀ shows void volume.

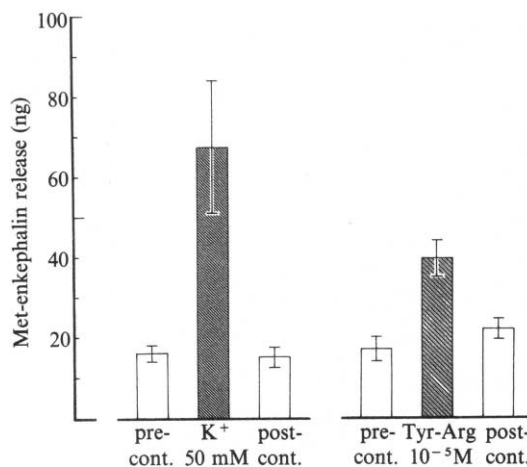


Fig. 4 Met-enkephalin release from rat striatal slices. Guinea pig striatal slices (500 μ m thick, sagittal section) from four striata taken from two animals weighing ~600 mg were used in each experiment. The slices were superfused at a rate of 1 ml min⁻¹ with a Krebs-bicarbonate medium at 37 °C, containing the dipeptides, Leu-Leu, Leu-Gly, Tyr-Tyr and Gly-Phe (each 1 mM) to protect against loss of released enkephalin by metabolism, and gassed with 95% O₂ and 5% CO₂. After an initial wash period of 15 min, superfusate samples were collected at 15-min intervals. For the first collection period, slices were superfused with normal medium containing the dipeptides (precontrol release). On the second collection period, slices were exposed to a high K⁺ (50 mM) or Tyr-Arg (10⁻⁵ M)-containing medium (stimulated output) and the medium was replaced with normal solution for a 15-min period (post-control release). For the determination of the released Met-enkephalin, the method described by Meek and Bohan¹⁰ was used. Final recovery of Met-enkephalin with this method was approximately 94%. The results are means $\pm$ s.e.m. for three or four experiments, as the absolute value of output from each striatal preparation.

In preliminary experiments, Tyr-Arg inhibited the enkephalin degradation enzymes in mouse brain homogenate preparations. However, its effect was weaker than that of bacitracin (less than 1/5th–1/10th, on a molar basis), which is a potent enkephalin degradation blocker. In addition, bacitracin did not produce analgesia even with intracisternal administration in a dose of 100 μ g per mouse (71 nmol per mouse).

Thus, the analgesic effect of Tyr-Arg is interpreted as follows. Tyr-Arg itself does not bind with the opiate receptors but acts to release Met-enkephalin, which does bind with opiate receptors effectively, and consequently produces a potent analgesia. The relatively long-lasting analgesia induced by Tyr-Arg may be attributed to a stabilising effect on the released Met-enkephalin because Tyr-Arg weakly inhibits enkephalin degradation enzymes. Thus, Tyr-Arg seems to be a Met-enkephalin releasing factor, and its presence suggests the existence of a regulating mechanism for the release of Met-enkephalin. A preliminary report of a part of this work has been made elsewhere¹¹.

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Stimulation of the pituitary-adrenocortical axis by nerve growth factor

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Nerve growth factor (NGF) is a protein essential for the development and maintenance of the peripheral sympathetic nervous system^{1,2}, causing responsive neurones to increase in size and to extend neurites. Biochemically, the selective induction of tyrosine hydroxylase (TH) and dopamine β -hydroxylase^{3,4} key enzymes in catecholamine biosynthesis, is one of its most characteristic effects. Both the morphological⁵ and biochemical⁶ effects are modulated by glucocorticoids, suggesting a close relationship between specific effects of NGF and hormone action. NGF has been shown to induce an increase in adrenal cyclic AMP in intact but not in hypophysectomised rats⁷, and so we have looked directly at the effect of systemic administration of NGF on the hypothalamo-pituitary-adrenal axis. We report here that NGF induced an enhanced secretion of adrenocorticotropin (ACTH) and a prolonged increase in plasma glucocorticoid concentration after intravenous (i.v.) injection. Such effects could have important implications for the biological activity of NGF.

NGF was prepared from submaxillary glands of adult male mice by the method of Bocchini and Angeletti⁸, as the 2.5S subunit, with the modifications of Suda *et al.*⁹. The purity of NGF was controlled by SDS gel electrophoresis and its biological activity was determined as described by Fenton¹⁰. The biological activity of our NGF preparation was 250 biological units per μ g of protein. Biologically inactive NGF was prepared by oxidation of the tryptophan residues with *N*-bromosuccinimide as described by Frazier *et al.*¹¹. Adult male Sprague-Dawley rats of 100–120 g body weight were maintained at 24 °C, five per cage, with lights on at 0700 h and off at 1900 h. Food and water were provided *ad libitum*. For hormone studies they were injected in the tail vein with NGF at between 0700 h and 0900 h. They were killed by decapitation and trunk blood was collected at intervals of 5–720 min. Plasma ACTH and corticosterone were determined by radioimmunoassay as previously described¹². The ACTH antibody used cross-reacts completely on a molar basis with ACTH_{1–24} but not (cross-reaction <0.005%) with α -melanocyte-stimulating hormone

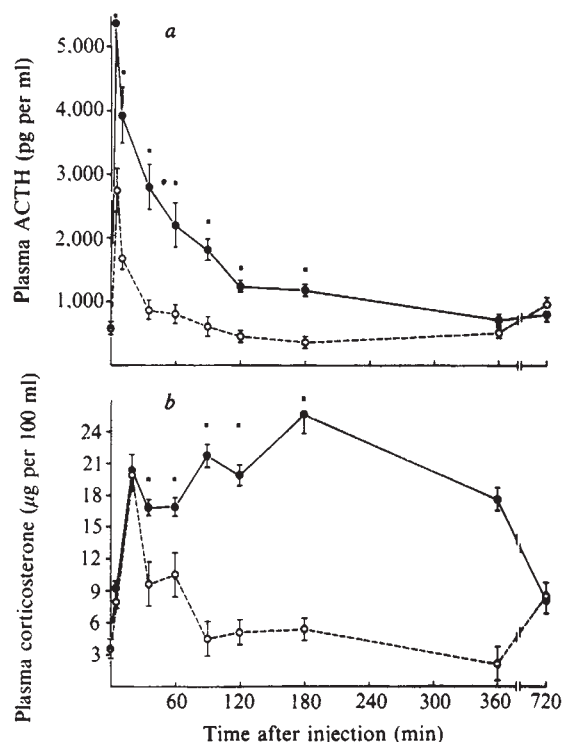


Fig. 1 Stimulation of the pituitary-adrenocortical axis by NGF. Groups of 5–10 rats were injected i.v. with 100 pmol per g NGF (●) or equimolar amounts of cytochrome *c* (○). They were killed at the times indicated and plasma was assayed for ACTH (a) and corticosterone (b) by radioimmunoassay¹². Values represent the mean $\pm$ s.e.m.

* $P < 0.0025$, compared with cytochrome *c* controls.

(α -MSH), β -MSH, ACTH_{17–39} or β -endorphin. There was no cross-reactivity with NGF. TH activity was determined according to Mueller *et al.*¹³.

A single i.v. injection of NGF (100 pmol per g) produced a prolonged stimulation of the adrenocortical system (Fig. 1). NGF increased plasma ACTH levels from 576 ± 110 pg ml⁻¹ in controls to $5,380 \pm 690$ pg ml⁻¹ ($n = 10$) at 5 min. ACTH did not return to resting levels within 180 min. Plasma corticosterone levels continued to increase for 20 min and remained at a high plateau of $20\text{--}24$ μ g per 100 ml for at least 6 h. The specificity of the response to NGF was confirmed by injecting control animals with equimolar concentrations of cytochrome *c*, a protein similar to NGF in molecular weight and isoelectric point. The

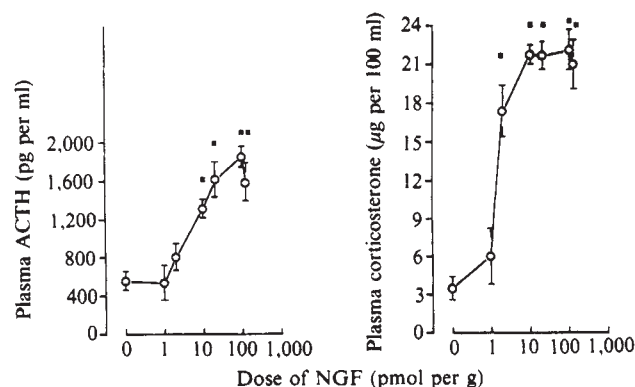


Fig. 2 Effect of concentration of NGF on stimulation of pituitary-adrenocortical axis. Groups of five rats were injected i.v. with NGF at 1.0–300 pmol per g. They were killed 90 min later and plasma was assayed for ACTH and corticosterone¹². Values are expressed as mean $\pm$ s.e.m.

* $P < 0.005$, compared with cytochrome *c* controls.

Table 1 Specificity of NGF for hormonal changes in rat plasma

	ACTH (pg per ml)	Corticosterone (μ g per 100 ml)
Controls	576 ± 110	3.5 ± 1.1
Saline	609 ± 142	4.1 ± 1.2
NGF	$1,223 \pm 113^*$	$17.5 \pm 1.8^*$
Cytochrome <i>c</i>	582 ± 138	4.7 ± 1.6
Inactive NGF	343 ± 145	3.1 ± 1.1

Groups of five rats were injected i.v. with saline or equimolar concentrations (10 pmol per g BW) of various proteins. Rats were killed 90 min later and plasma ACTH and corticosterone were measured¹². Results are mean $\pm$ s.e.m.

* $P < 0.005$, compared with controls (saline or cytochrome *c*) or with inactive NGF.

animals showed only hormonal changes of short duration (Fig. 1): plasma ACTH increased ($2,750 \pm 349$ pg ml⁻¹, $n = 10$) at 5 min, returning to resting levels within 35 min, and plasma corticosterone levels peaked at 20 min, returning to normal within 90 min. Thus the systemic application of NGF causes prolonged stimulation of the pituitary-adrenocortical axis that clearly differs from the acute short-lasting response to cytochrome c.

Injection of biologically inactive NGF had no significant effect on plasma ACTH or corticosterone levels (Table 1), indicating that only the biologically active molecule can activate the pituitary-adrenocortical axis. The hormonal response to biologically active NGF is dose-dependent (Fig. 2). The minimum dose that resulted in a significant ($P < 0.005$) increase in plasma ACTH and corticosterone 90 min after injection was 10 pmol per g. Maximum effect was achieved with concentrations 10 times greater.

Table 2 Effect of NGF on TH activity in sympathetic ganglia of rats

Treatment	TH activity (nmol dopa per h per mg protein)	
	Superior cervical ganglion	Coeliac ganglion
Saline	1.2 $\pm$ 0.06	0.95 $\pm$ 0.12
NGF	1.71 $\pm$ 0.15*	2.51 $\pm$ 0.17†
Inactive NGF	1.0 $\pm$ 0.05	1.01 $\pm$ 0.05

Groups of six rats were injected i.v. with saline or equimolar concentrations (10 pmol per g) of biologically active or inactive NGF. TH activity was determined 48 h later¹³ by the amount of 3,4-dihydroxyphenylalanine (dopa) formed.

* $P < 0.025$ } Compared to animals treated with inactive NGF or
† $P < 0.01$ } saline.

The minimum dose of NGF (10 pmol per g) required to induce hormonal changes in plasma is identical to that necessary to elicit a significant ($P < 0.025$) induction of TH in sympathetic ganglia (Table 2). Thus NGF-mediated hormonal changes could have important implications for the initiation of selective induction of TH activity in sympathetic neurones. We do not know whether these hormonal changes are of peripheral or central origin. However, the short latency of the increase in plasma ACTH may indicate direct action on the hypothalamo-pituitary system, as suggested by the observations of Nagaiah *et al.*¹⁴. Our findings, together with the observation that intracerebral injection of NGF induces an increased drinking response^{15,16}, indicate that NGF can affect both central and peripheral nervous systems. The significance of the hormonal changes with respect to the physiological role of NGF remains to be elucidated.

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Evidence for thyroxine–growth hormone interaction during brain development

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Growth-promoting hormones have complementary and synergistic effects on cell growth. In particular, thyroid hormone–growth hormone (GH) interactions are important in the regulation of somatic development¹. Although the requirement of thyroid hormone for normal brain development is well established², the effects of growth-promoting peptide hormones, including GH, nerve growth factor and insulin, on central nervous system development have not been well characterised. To investigate the possibility of a role for GH in normal CNS development, we have measured its effect on the activity of brain ornithine decarboxylase (ODC), a key enzyme in the synthesis of the polyamines, spermidine and spermine^{3,4}. Previously, we reported increased ODC activity in neonatal rat brain after intraperitoneal (i.p.) or intracranial administration of GH and other growth-promoting peptide hormones⁵, results which have been supported by other data^{6,7}. We now extend our findings to show that GH stimulation of brain ODC activity depends on the presence of thyroid hormone, even though thyroid hormone, by itself, does not stimulate ODC activity in brain. This is in contrast to liver, where GH and thyroid hormones seem to stimulate ODC activity independently. Our results suggest that thyroid hormone selectively alters or modulates the action of GH in mammalian brain and that abnormal brain development observed in hypothyroidism may in part result from decreased expression of GH activity.

One-day-old Sprague–Dawley rats (Zivic–Miller, Allison Park, Pennsylvania) were thyroidectomised (thyrx) by treatment with 100 μ Ci of Na¹³¹I (ref. 8). At 18 d, normal and thyrx rats were injected with saline (control), thyroxine (T₄), bovine GH (bGH), or T₄ and bGH (for details see Table 1 legend). ODC activity was determined *in vitro* as ¹⁴CO₂ produced from [¹⁴C]ornithine^{3,6}. At 18 d, body and brain weights of the thyroid-deficient rats were significantly reduced compared with controls; the body weights of thyrx and control rats averaged 37.5 $\pm$ 1.7 g (mean $\pm$ s.e.m.) and 42.7 $\pm$ 0.5 g ($P < 0.05$), respectively, and respective brain weights averaged 1.43 $\pm$ 0.03 g and 1.58 $\pm$ 0.03 g ($P < 0.02$).

In normal rats, i.p. bGH stimulated brain ODC activity twofold (217% of control, Table 1, expt 1), a finding similar to that observed in previous studies⁵. Although T₄ alone did not significantly increase brain ODC activity (expt 2), stimulation by T₄ and bGH given together was equivalent to that by bGH alone (expt 1).

The basal ODC activity of thyrx control (saline-treated) rats was elevated (158%) compared with normal controls (expt 2). This may have resulted from post-thyroidectomy delay in the normal decline of ODC activity from high perinatal to low adult levels¹⁰, similar to the hypothyroidism-induced delay of brain maturation observed by others². As in normal animals, brain ODC activity of thyrx rats was not significantly altered by T₄ treatment (129 $\pm$ 17% of thyrx controls). Importantly, bGH also failed to increase ODC activity in thyrx rats, and administration

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Table 1 Effects of thyroxine and thyroidectomy on bGH stimulation of brain ODC activity

	Treatment	ODC activity	% of control	<i>P</i>	
Expt 1 Normal rats	Control	722 ± 28 (3)	—	—	
	bGH	1,568 ± 211 (4)	217 ± 29	<0.02	
	T ₄ + bGH	1,464 ± 159 (5)	203 ± 22	<0.001	
Expt 2 Normal rats	Control	817 ± 83 (8)	—	—	
	T ₄	1,036 ± 92 (5)	127 ± 11	NS	
	Thyrx rats	Control	1,294 ± 122 (7)	158 ± 15*	<0.01*
		T ₄	1,663 ± 218 (8)	129 ± 17	NS
		bGH	1,391 ± 146 (9)	108 ± 11	NS
T ₄ ± bGH		2,844 ± 196 (9)	220 ± 15	<0.001	

Normal and thyroidectomised (thyrex) rats received saline (control), T₄ (20 µg per 100 g body weight) i.p. at 28, 19 and 4 h before death, bGH (300 µg per 100 g body wt) i.p. 4 h before death, or T₄ plus bGH. bGH (NIH-GH-B16) was chromatographed before use to obtain a monomer fraction²⁰. ODC activity (d.p.m. per 30 min per g wet weight) is given as mean ± s.e.m., with the number of animals in parentheses. NS, Not significant.

* Compared with normal control.

of T₄ before bGH completely restored the ODC response, with an activity increased to 220% of control levels. This was comparable to the normal response to bGH (217% of control) seen in euthyroid rats.

This effect of T₄ was not apparent in liver (Table 2). As noted by us and others^{5,11}, GH treatment of normal rats produced a large (148-fold) increase in liver ODC activity (expt 1). T₄ increased liver ODC activity eightfold in normal and thyrex rats (expt 2), and the increase seen after T₄ and bGH in normal rats did not differ from that of bGH alone (expt 1). In contrast to results with brain, liver ODC activity in thyroid-deficient rats was stimulated by bGH (37-fold increase, expt 2). Furthermore, the response of T₄ plus bGH (48-fold increase) was similar to that for bGH alone. As shown in Table 2, after bGH, or T₄ plus bGH, the stimulation of ODC activity was greater in normal rats than in thyrex rats. This may reflect a decrease in liver responsiveness to GH after thyroidectomy which was not adequately corrected by T₄ administration. It is clear, however, that in contrast to its effect on brain, GH stimulated liver ODC activity in thyrex rats without T₄ pretreatment.

We conclude that an action of T₄ is required for the stimulation of brain ODC by bGH. The mechanism for this 'permissive' (ref. 12) or modulating action of T₄ is unknown. It is possible that the number of receptors for GH or for a GH-dependent agent such as somatomedin¹³ is reduced in thyroid deficiency in a manner similar to that reported for prolactin¹⁴ and catecholamine receptors¹⁵. Alternatively, thyroxine may regulate the availability of GH-dependent (perhaps brain-specific) somatomedin or somatomedin-like factors. The difference between brain and liver response might also result from

differential effects of T₄ on ODC isoenzymes, the existence of which is suggested by kinetic data¹⁶.

Because, in severe hypothyroidism, the amount of GH in plasma is greatly reduced¹⁷, attempts have been made to correct the GH deficiency in thyroid-deficient neonatal rats with large amounts of exogenous hormone^{18,19}. Interestingly, this treatment partially reversed the abnormal pattern of neonatal brain growth. Thus, in neonatal hypothyroidism, abnormal brain development might result in part not only from decreased GH or GH-dependent growth factors, but also, as suggested by the present study, from a lack of thyroid hormone support for the limited GH present.

As ODC activity and polyamine synthesis seem to be intimately associated with the regulation of nucleic acid and protein synthesis^{3,9}, the increase in brain ODC activity following GH administration suggests a role for GH in the regulation of brain development. It should be recognised, however, that in this and previous work⁵, ODC activity has been used as an indicator of growth stimulation³. It remains to be determined whether the regulatory actions of GH on brain development are mediated by changes in polyamine synthesis.

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Apamin blocks certain neurotransmitter-induced increases in potassium permeability

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Apamin¹ is a neurotoxic polypeptide of known structure^{2,3} isolated from bee venom. Shuba and coworkers^{4,5} have recently shown that it abolishes the hyperpolarising action of externally-applied ATP on visceral smooth muscle (guinea pig stomach and taenia coli) as well as the hyperpolarisation (inhibitory junction potential) that follows stimulation of the non-adrenergic inhibitory nerve supply to these tissues. As it has been proposed⁶ that

Table 2 Effects of thyroxine and thyroidectomy on bGH stimulation of liver ODC activity

	Treatment	ODC activity	<u>Treated</u> Control	P
Expt 1	Control	483 ± 186 (3)	—	—
	Normal rats bGH	71,343 ± 8,184 (4)	148-fold ± 17	<0.001
	T ₄ ± bGH	57,691 ± 8,879 (5)	119-fold ± 18	<0.001
Expt 2	Control	403 ± 117 (5)	—	—
	Normal rats T ₄	3,005 ± 581 (4)	8-fold ± 1	<0.01
	Thyrex rats Control	692 ± 271 (5)	1.7-fold ± 1*	NS*
	T ₄	5,715 ± 2,168 (5)	8.3-fold ± 2	<0.05
	bGH	25,707 ± 6,181 (5)	37-fold ± 9	<0.01
	T ₄ + bGH	33,288 ± 7,642 (5)	48-fold ± 11	<0.001

Details same as for Table 1.

ATP is the neurotransmitter involved in the latter response, Vladimirova and Shuba⁶ tentatively concluded that apamin is a specific postsynaptic blocking agent of this non-adrenergic, possibly 'purinergic', inhibition. We have confirmed the important observation that nanomolar concentrations of apamin reduce inhibition by ATP and by non-adrenergic nerve stimulation, but further experiments suggest that, rather than acting as a specific blocker of ATP receptors, apamin inhibits the increase in potassium permeability caused by a number of agents, including ATP.

The first new finding (Fig. 1a) was that apamin also reduces the inhibition produced by stimulation of the sympathetic (perivascular) nerve supply to the taenia coli. As the transmitter in this case is noradrenaline⁷, it seemed possible that apamin interferes with some of the actions of catecholamines as well as of ATP. In keeping with this, inhibition of the taenia by the selective α -adrenoreceptor agonist amidephrine is also blocked by apamin. Figure 1b shows the noncompetitive nature of this antagonism, the maximum inhibition by amidephrine being reduced by apamin at concentrations as low as 1–3 nM. In each of four other such experiments, 5 nM apamin was sufficient to abolish amidephrine inhibition. These results contrast with the competitive antagonism seen with α -adrenoreceptor blockers in this tissue⁸, and suggest that apamin acts at a stage beyond the recognition site of the receptor. As both α -adrenoreceptor-mediated inhibition^{9–11} and the inhibitory response to ATP^{12,13}, as well as to non-adrenergic nerve stimulation¹⁴ are due mainly to an increase in the potassium permeability of the smooth muscle membrane, it seemed possible that apamin could act by preventing the rise in potassium permeability rather than by blocking the receptors that initiate it.

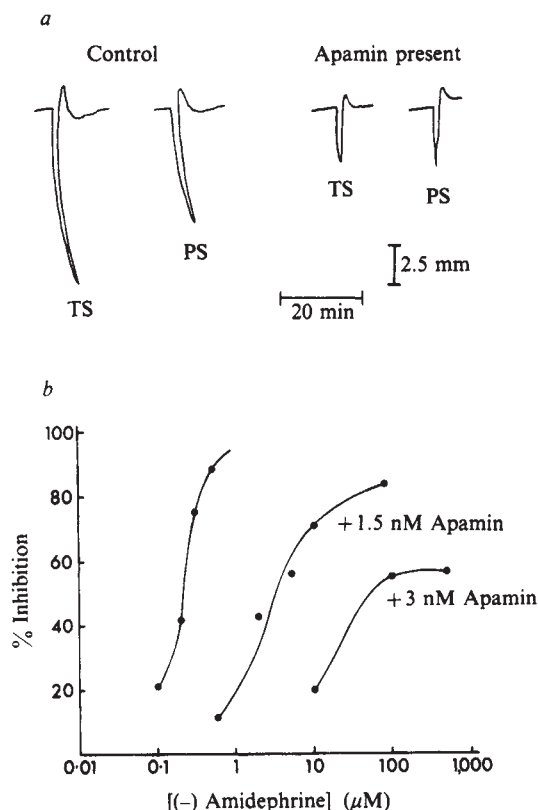


Fig. 1 a, Apamin (5 nM) reduces the relaxation caused by both transmural (TS) and perivascular (PS) stimulation of the nerve supply to an isolated strip of guinea pig taenia coli. Atropine (3 μ M) present throughout. Stimulation parameters: supramaximal square pulses (200 μ s) for 30 s at either 5 (TS) or 20 Hz (PS). Isotonic recording. b, Apamin antagonises the inhibition by the α -adrenoreceptor agonist amidephrine of the contractile response of a taenia strip to carbachol (20 nM). For details of the experimental procedures for a and b, see refs 7 and 8, respectively.

This hypothesis was tested using another tissue, guinea pig liver, in which α -adrenoreceptors also enhance potassium permeability, as indicated by increases in potassium flux, membrane potential and conductance, and in addition a net loss of potassium^{15,16}. ATP also hyperpolarises guinea pig liver cells, probably by the same mechanism¹⁷. The response can be conveniently studied using a suspension of isolated hepatocytes, as the loss of potassium following a substantial increase in potassium permeability is readily detected by means of a potassium-sensitive electrode in the suspension^{16,18}. This is shown in Fig. 2, which also shows that apamin greatly reduces the potassium loss, and hence, by inference, the increase in potassium permeability caused by both ATP and noradrenaline. In this respect apamin resembles quinine¹⁸, which is, however, approximately 10^5 times less active.

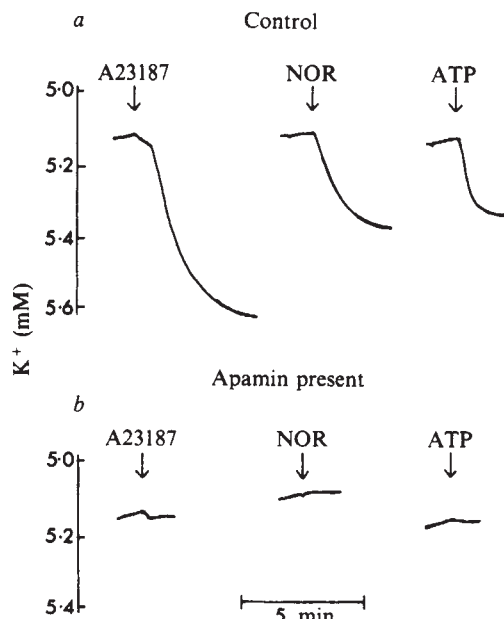


Fig. 2 a, A23187 (5 μ M), (-)noradrenaline (5 μ M, in the presence of propranolol at 5 μ M) and ATP (1 μ M) cause K loss from isolated guinea pig hepatocytes. The movement of K between the cells and the incubation medium was followed by means of a K-sensitive electrode in the cell suspension (which contained ~50 mg cells in 2 ml and was stirred). A downward deflection indicates an increase in external K concentration, corresponding to loss of K from the cells. For other details, see ref. 18. b, Apamin (10 nM, applied 2–3 min beforehand) greatly reduces the response to all three agents applied to other samples from the same batch of hepatocytes.

The rise in potassium permeability is thought^{18–20} to be triggered by an increase in the concentration of ionised calcium in the cytosol of the hepatocytes, and this suggested a means of testing whether apamin blocks the receptors or the consequences of receptor activation. If the former is the case, the change in calcium movement should also be abolished. This was studied using noradrenaline as the agonist, and the results are shown in Fig. 3. Apamin at concentrations sufficient to eliminate the potassium movement induced by noradrenaline caused little change in the rapid calcium loss that follows the application of α -agonists to hepatocytes^{21,22}. Thus the receptors are still functional. The same dissociation between potassium and calcium movements was seen with the divalent ionophore A23187, which in the presence of apamin no longer caused potassium loss (Fig. 2) although still initiating calcium movement¹⁸. A further test of the same point was made with rabbit liver slices which respond to α -agonists with increases in 45 Ca and 42 K efflux and a rise in glucose release¹⁹. Apamin (10 nM)

abolished the effect on ^{42}K efflux, whereas the changes in ^{45}Ca efflux and glucose release were unaltered.

The conclusion that apamin is not a receptor blocker is strengthened by our finding that it does not inhibit the contractile response of the isolated rat vas deferens preparation to ATP or to α -agonists. Nor are the actions of ATP on histamine release from rat peritoneal mast cells impaired (B. Gomperts, personal communication).

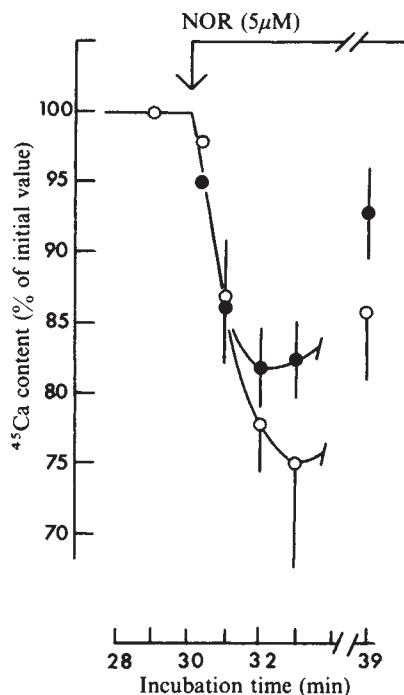


Fig. 3 Noradrenaline ($5\text{ }\mu\text{M}$, in the presence of propranolol at $5\text{ }\mu\text{M}$) causes isolated guinea pig hepatocytes to lose calcium, and the initial loss is little affected by apamin. Open circles, controls; closed circles, cells treated with apamin at either 10 (4 expts) or 100 nM (1 expt), applied 3 min before noradrenaline. Calcium contents were determined by equilibrating the hepatocytes with ^{45}Ca (total external calcium, 1.8 mmol l^{-1}) for 30 min and then counting the ^{45}Ca content of the pellet after centrifuging the cells through an oil phase, as described in ref. 18. The values (ordinate) have been expressed as percentages of the content before noradrenaline (mean value, 1.91 ± 0.08 (s.e.m., $n = 5$) nmol calcium per mg dry weight). The vertical bars indicate the s.e.m.s of 4–5 observations. Abscissa, incubation time in ^{45}Ca .

It is also clear that apamin is highly selective in that only some potassium permeabilities are blocked. Thus Vladimirova and coworkers have shown⁵ that concentrations of apamin much greater than those used for their smooth muscle experiments have little effect on the frog neuromuscular junction, or on synaptic inhibition in an identified molluscan neurone. Other actions that we have found to be unaffected by apamin at up to micromolar concentrations include: (1) the inhibitory effect of acetylcholine on the beat of the isolated perfused rabbit heart; (2) the increase in potassium permeability that follows a rise in intracellular calcium in human red blood cells; and (3) inhibition of the mechanical activity of the guinea pig taenia coli by β -adrenoreceptor agonists and by low concentrations of adenosine and AMP. This last finding is in keeping with other evidence (see refs 23, 24) that inhibition of the taenia coli by β -adrenoreceptors, as well as by the sub-type of purinoceptor which is activated by adenosine and by AMP, does not involve the increase in potassium permeability seen with α -adrenoreceptor agonists and ATP.

In conclusion, apamin abolishes certain responses to ATP and to α -adrenoreceptor agonists by inhibiting the increase in

potassium permeability that these agents initiate. The simplest explanation is that apamin blocks the potassium channels involved, although it is also possible that it interferes with the mechanism by which a rise in cytosolic calcium causes these channels to open. The potency and selectivity of apamin should make it useful for the study of neurotransmitter-controlled potassium conductances. In addition, the finding that apamin blocks calcium-mediated increases in the potassium permeability of hepatocytes but not red blood cells suggests that there may be several types of calcium-dependent potassium channel.

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Induction and kinetics of natural killer cells in humans following interferon therapy

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Natural killer (NK) cells are non-B, non-T lymphocytes that effect spontaneous cytotoxicity of both virus-infected and neoplastically transformed target cells^{1,2}. These NK lymphocytes have been detected in several species including man^{3–9}. Interferon is a primary regulator of natural killer activity^{6,10,11}. Because NK cells have been implicated in the regulation of tumour cell expression and can be induced by interferon in murine models, we have studied patients receiving large doses of interferon to determine (1) whether interferon could induce NK lymphocytes in the peripheral blood of man, and (2) whether there are characteristic kinetics for the appearance, disappearance and reactivation of NK lymphocytes following interferon therapy.

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We report here the activation of human NK cells by the systemic inoculation of human subjects with interferon. Five patients received interferon as therapy for non-Hodgkin's lymphoma. All showed a marked increase in NK cell activity 12–24 h after inoculation. Peak NK activity occurred 18 h after introducing interferon, and thereafter declined rapidly but remained above pre-interferon levels. Induced NK activity occurred with reinroduction of interferon but at lower levels of activity and with different kinetics.

The interferon used in this study was from a human leukocyte interferon preparation containing 1×10^6 units of interferon per mg protein. Patients receiving interferon had non-Hodgkin's lymphoma. (For more details about the interferon preparation, its administration, the diagnostic criteria and clinical course of the non-Hodgkin's lymphoma patients, see ref. 12.) Four (W.M., P.K., B.S., J.B.) of the patients were treated with 10×10^6 units of interferon intramuscularly for 7–30 days, and one patient (G.L.) received 5×10^6 units for 2 days, followed by 10×10^6 units daily for the next 27 days. Of these patients only P.K. had received interferon previously. Data for NK activity are expressed in terms of the cytotoxicity index (CI). This allows each patient to act as his own control throughout all observations. The CI was determined by dividing the specific killing of MOLT-4 target cells at a given experimental time by the maximum killing of MOLT-4 target cells observed for that patient's lymphocytes during the entire course of observations. The CI values for each of the patients before interferon therapy were 0.16, 0.08, 0.02, 0.14 and 0.5, respectively (Fig. 1). Lysis was detected in a 6-h ^{51}Cr release assay of triplicate samples, with variance among triplicates of less than 10%. Lymphocytes from the two patients (W.M. and P.K.) with the highest CI values had been tested against various targets including MOLT-4 cells, HeLa cells uninfected or persistently infected with measles virus, HT-29 cells and Daudi cells. The characterisation, handling, passage and ^{51}Cr -labelling of these cells have been reported elsewhere^{13,14}. Killing by lymphocytes from several normal controls and from these two patients was effective against the various targets, but more MOLT-4 cells were lysed than other target strains, and so thereafter, only ^{51}Cr -labelled MOLT-4 cells were used. These cells ($1-2 \times 10^4$ cells ml^{-1}) were mixed with varying amounts of lymphocytes collected from each patient's peripheral blood by Ficoll-Hypaque passage, depleted of adherent cells¹⁴, and used at ratios of 1:10, 1:20 and 1:40. To ensure internal consistency throughout the various time points assayed, mean values of spontaneous ^{51}Cr release (range 10–23%) were calculated and results corrected at each point as described¹⁴.

As shown in Fig. 1, enhanced NK lymphocyte activity was not present at 6 h, but appeared at 12 h and peaked at 18 h after the initial injection of interferon. Thereafter, killing activity fell rapidly but stayed above pre-interferon levels. Lymphocyte killing generated by interferon therapy was caused by lymphocytes which expressed Fc receptors. Patients (W.M. and P.K.) had less than 1% of the total killing activity in Fc-negative lymphocytes. Mean specific ^{51}Cr release $\pm$ 1 s.d. due to total peripheral blood lymphocytes compared with adherent cell-depleted Fc-positive and Fc-negative populations was: patient W.M., total lymphocytes $44 \pm 6\%$, Fc positive, $32 \pm 1\%$, Fc negative $3 \pm 0.3\%$; patient P.K., total lymphocytes $28 \pm 6\%$, Fc positive $17 \pm 0.2\%$, Fc negative, 0%. Thus, because (1) lymphocytes bearing Fc-positive receptors performed the killing, (2) many different target cell lines were lysed, and (3) cytotoxicity was induced by interferon, these killer cells can be characterised as NK lymphocytes^{1,2,15,16}.

We were interested in whether NK lymphocyte killing responses could be restimulated in patients who had previously been given interferon therapy. Two patients (J.B., B.S.) received 10×10^6 units interferon daily for 7 days, and were taken off therapy for 46 and 73 days, respectively. They were then given 10×10^6 units interferon daily for 9 or 30 days, respectively. In patient B.S., whose CI was 0.1, the initial course of interferon increased NK activity to a CI of 1.0 by 18 h. However, the

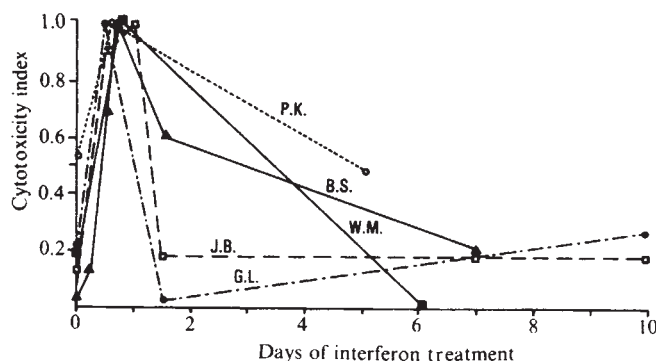


Fig. 1 The cytotoxic indices of peripheral blood lymphocytes (PBLs) from five patients treated with 10×10^6 units interferon daily plotted as a function of time. Peripheral blood lymphocytes, sampled before interferon therapy and at the indicated intervals thereafter, were assayed for spontaneous cytolytic activity against MOLT-4 cells in a standardised 6-h ^{51}Cr -release assay at lymphocyte: target ratios of 5:1, 20:1 and 40:1. The cytotoxic indices were calculated from % specific killing data at a PBL: target ratio of 40:1 by dividing the % specific killing at a given time by the maximum % specific killing observed for that patient during treatment. % Specific killing data represented the mean of at least three replicates and were corrected for variance in the spontaneous releases, which ranged from 6 to 24%. Cytotoxic indices for normal subjects assayed concurrently had a mean of 0.9 and the minimum CI seen in a single normal subject was 0.5.

second interferon course generated no NK activity. Patient J.B. did develop NK lymphocyte activity 73 days after reinoculation with interferon (CI 0.2), but at fivefold lower levels than those generated during the primary inoculation of interferon. When interferon therapy was attempted a third time in patient J.B. (10×10^6 units of interferon given daily for 30 days) 150 days after cessation of the second course, a significant rise in NK activity was seen (CI 0.5). Further, the elevated NK response observed in P.K. (Fig. 1) to interferon occurred 1.5 yr after a prior course of leukocyte interferon. Throughout these studies there was no extension of the patients' lymphoma and no introduction of chemotherapy. Therefore, neither haematological complications of conventional chemotherapy nor a worsening of their disease can explain the transient refractoriness of B.S. and J.B. to repeated interferon induction.

Recently, patients with non-Hodgkin's lymphoma underwent remission in both size and distribution of their lymphadenopathy after intensive therapy with interferon¹². Prevention of relapse has also been reported in patients with osteosarcoma receiving interferon¹⁷. We have noted the successful induction of NK lymphocyte activity in patients with non-Hodgkin's lymphomas following interferon treatment. Hence, in humans, like mice, interferon induces NK activity. The role of induced NK activity against the patient's own tumour cells is now under investigation.

After completion of these studies, we became aware that Einhorn, Blomgren and Strander¹⁸ had also demonstrated acute enhancement of NK responses in four of five tumour patients given a single injection of 3×10^6 units of human leukocyte interferon.

The interferon used was obtained from Dr Kari Cantell, Finnish Red Cross Transfusion Service. This research was supported by USPHS research grants NS-12428, AI-07007, AI-09484 and CA-16857-03 and Biomedical Research Support Program grant 1 S07-RR-05514. J.R.H. is a recipient of a postdoctoral fellowship from the Multiple Sclerosis Society.

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Desensitisation of cultured glial cells to epidermal growth factor by receptor down-regulation

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Epidermal growth factor (EGF), which can be purified from the mouse submaxillary gland¹ or from pregnant human urine², is a potent multiplication-stimulating factor for several types of cultured cells, including human fibroblasts^{2,3} and glial cells⁴. The molecule binds with high affinity and saturation kinetics to a cell-surface receptor⁵, is subsequently internalised^{5,7} and finally degraded^{5,6}. The binding event is accompanied by a reduction in the number of EGF receptors^{5,8}. This phenomenon—'receptor down-regulation'—has been demonstrated with several hormones and may be a general principle for the modulation of binding groups on the outer cell surface^{9,10}. Further, it has been proposed that receptor loss acts to regulate the cellular response to the binding ligand¹¹. The present study provides direct experimental support for this hypothesis. It demonstrates that down-regulation of EGF receptors on glial cells causes desensitisation of the mitogenic response of these cells to subsequent stimulation with EGF.

Attempts to demonstrate cellular desensitisation, that is, a decreased metabolic response to a factor, may be hampered by the fact that the initial exposure to the factor is likely to produce significant changes in the basal level of the metabolic parameter in question. The present experiments were designed to dissociate the event of down-regulation of EGF receptors on glial cells from the mitogenic response of these cells to EGF. Treatment of glial cells with increasing concentrations of EGF (up to 167 nM) in Ham's F-10 medium for 24 h resulted in progressive down-regulation of the EGF receptors; loss of about 90% of the

EGF receptors could be induced, as revealed by Scatchard analysis¹² of binding experiments with ¹²⁵I-labelled EGF (Fig. 1). This finding was expected by analogy with previous data on EGF receptors of other types of cultured cells^{5,8}. In contrast, EGF was incapable of inducing a mitogenic response in the presence of plain F-10 medium; only after supplementation with human serum albumin (HSA) was this medium permissive to multiplication-stimulating activity (Table 1). Pre-exposure of glial cells to plain F-10 medium did not affect the time (about 13 h) required for subsequent recruitment of cells to DNA synthesis on stimulation with EGF in the permissive medium (F-10 medium supplemented with HSA) (Fig. 2). This suggests that the block in the mitogenic response to EGF imposed by the non-permissive conditions was probably at an early step close to the ligand–receptor binding event and preceding the reactions leading to commitment of the cells to enter the cell cycle.

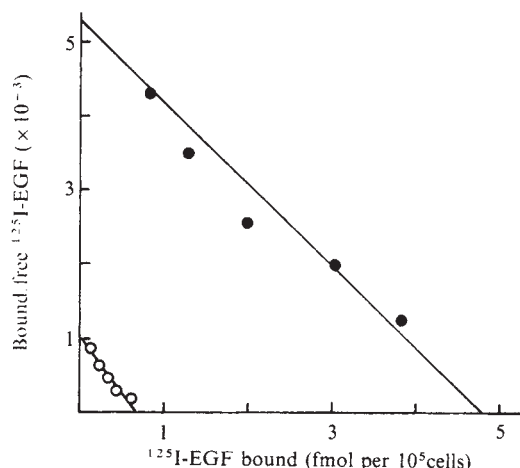


Fig. 1 Effect of EGF pretreatment on ¹²⁵I-EGF binding to human cultured glial cells. Human normal glia-like cells, line 787 CG (refs 16, 17), were seeded on 2.5-cm dishes in 2 ml of Eagle's medium containing 10% fetal calf serum. After 48 h at 37°C the medium was replaced with 2 ml of Ham's F-10 without serum. The cultures (approximately 1.1×10^5 cells per dish) were then incubated for 22 h in 2 ml of Ham's F-10 medium in the absence (●) or presence of 167 nM EGF (○). Cells were then washed once with 2 ml of plain F-10 medium and five times with F-10 medium containing 1 mg ml^{-1} of HSA. The washed cells were incubated for 2 h at 4°C with various doses of ¹²⁵I-EGF in 1 ml of F-10 medium containing 1 mg ml^{-1} HSA. The cultures were then washed six times with 2 ml of ice-cold phosphate-buffered saline (137 mM NaCl, 2.7 mM KCl, 8 mM Na₂HPO₄, 1.5 mM KH₂PO₄, 0.9 mM CaCl₂, 0.8 mM MgSO₄, pH 7.3) containing 1% fetal calf serum. Cells were lysed in 1 ml of 0.3 M NaOH and the cell-bound radioactivity determined in a Packard Auto-Gamma scintillation spectrometer. Nonspecific binding, determined in the presence of excess unlabelled EGF (about $1 \mu\text{M}$) was subtracted. Binding data were treated by Scatchard analysis¹².

Table 1 Multiplication-stimulating activity of EGF on glial cells in F-10 medium, with or without human serum albumin

Addition	Multiplication-stimulating activity (c.p.m. ³ H)
None	814
EGF (1.6 nM)	1,121
HSA (1 mg ml^{-1})	2,716
HSA (1 mg ml^{-1}) + EGF (1.6 nM)	10,926

Multiplication-stimulating activity was estimated using the incorporation of ³H-thymidine into trichloroacetic acid-precipitable material of serum-deprived sparse cultures of a human normal glia-like cell line^{16,17} as described elsewhere¹⁸.

Cell cultures in which the EGF receptor number had been reduced by preincubation with EGF (in non-permissive medium) were less responsive to further exposure to EGF (in the permissive medium). The dose–response curve for EGF (based on the increase in labelling index) was more than fivefold shifted to the right in these cells compared with controls with normal EGF receptors (Fig. 3). The maximal response obtained at saturating concentrations of EGF was only slightly lower than in control cultures. Apparently, the post-receptor pathway in EGF-induced mitogenesis did not become limiting by preincubation with EGF; rather, desensitisation occurred by reduction in receptor number. Maximal biological response, demonstrable with only a fraction of the total number of receptors occupied, indicates the presence of spare receptors for EGF on glial cells, in accordance with previous data¹³.

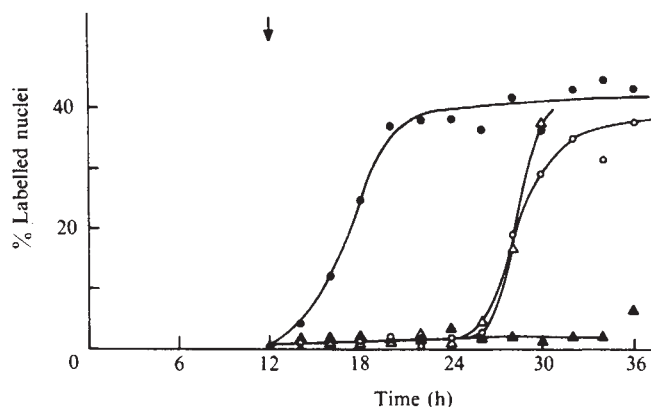


Fig. 2. Effect of EGF pretreatment on the time required for recruitment of glial cells to DNA synthesis on stimulation with EGF. Human normal glia-like cells, line 787 CG (refs 16, 17), were seeded on 2.5-cm dishes in 2 ml of Eagle's medium, containing 10% fetal calf serum ($\sim 10^5$ cells per dish). After 24 h at 37 °C the medium was replaced with 2 ml of Ham's F-10 medium without serum. After another 24 h, medium was changed to fresh F-10 medium containing $0.1 \mu\text{Ci ml}^{-1}$ ^3H -thymidine (specific radioactivity 2 Ci mmol^{-1}). The cultures were then given EGF (1.67 nM) and HSA (1 mg ml^{-1}) at the following times: EGF and HSA at 0 h (●); EGF at 0 h and HSA at 12 h (○); EGF and HSA at 12 h (△); EGF only at 0 h (▲). At the indicated times duplicate dishes were collected for autoradiography as described elsewhere^{16,18}.

hormone receptors¹⁵. These results differ from the present ones in some quantitative aspects, but together they suggest that modulation of receptor number may be a general mechanism for the regulation of cellular responsiveness to hormones.

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Adipokinetic hormone inhibits protein synthesis in *Locusta*

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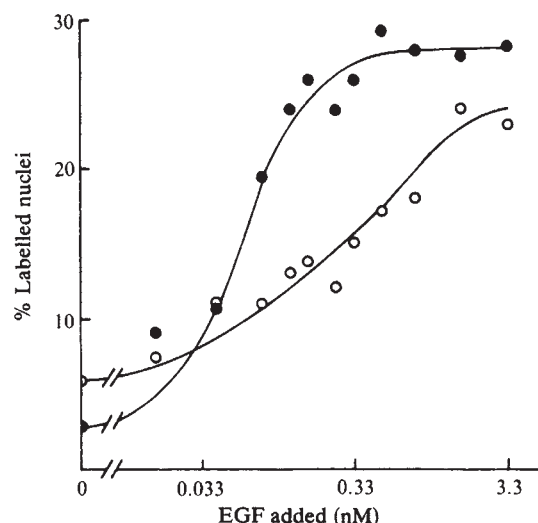


Fig. 3. Effect of EGF receptor down-regulation on the mitogenic response of human cultured glial cells to EGF. Glial cells were seeded as described in Fig. 1. After 24 h at 37 °C the medium was changed to Ham's F-10 without serum and the incubation continued for another 24 h. The cultures were then given 2 ml of fresh F-10 medium (●) or 2 ml of F-10 medium containing 167 nM EGF (○). After 22 h the cells were washed once with 2 ml of plain F-10 medium and five times with F-10 medium supplemented with HSA (1 mg ml^{-1}) and incubated in 2 ml of this medium containing various doses of EGF. After 24 h $0.4 \mu\text{Ci}$ of ^3H -thymidine were added and incubation continued for another 60 min. Cultures were then collected for autoradiography^{16,18}. Each point represents the average of duplicate dishes.

The adipokinetic hormone (AKH) of the locust has been isolated, characterised and synthesised^{1,2}. In this insect, its function is to mobilise diglycerides from the fat body to provide energy for sustained flight³. In addition, it modifies the metabolic activity of the flight muscles so as to oxidise fatty acids preferentially⁴. We report here another effect of AKH on locust metabolism, the direct inhibition of protein synthesis by the fat-body. This inhibitory activity may function in the locusts' overall adaptation to flight and it may also account for the presence of AKH both in larval locusts⁵ and in other species which do not fly for prolonged periods⁶.

There have been several studies of the neuroendocrine control of protein synthesis in the locust. Hill⁷ has shown that electrocautery of the median neurosecretory cells (MNSC) of the brain brought about a diminution of protein synthesis. Osbourne *et al.*⁸ reported that injection of homogenates of corpora cardiaca (CC) stimulated protein synthesis in adult female locusts. In larval locusts alterations in protein synthetic activity may be correlated with changes in ecdysone titre^{9,10}. Because the prothoracic gland is under neuroendocrine control in the larval insect the neurosecretory control of protein synthesis may be indirect though the mode of control in the adult insect is not known. To investigate this problem further, we studied the role of the CC in protein synthetic activity. The locust CC presents an unusual opportunity for the study of insect neurohormones because it can be separated into 'storage' and 'glandular' lobes¹³. Activity was tested by injecting CC homogenates into 5-d-old fifth instar larvae together with $1 \mu\text{Ci}$ ^3H -leucine (Amersham/Searle specific activity $50.6 \text{ Ci mmol}^{-1}$). Control insects received an equivalent

injection containing a homogenate of the optic lobe of the brain. After 2 h the animals were bled through the cervical membrane and the incorporation of ^3H -leucine into haemolymph protein was determined¹². When homogenates of whole cardiaca were injected (one gland in 10 μl saline) there was no consistent difference in incorporation of label into haemolymph protein between experimental and control insects.

However, when each lobe was homogenised separately, the injection of glandular lobe homogenates suppressed the incorporation of labelled amino acid into haemolymph protein by 24% compared with controls. A factor was found in extracts of the storage lobe that stimulated protein synthesis. The nature of this enhancement of protein synthesis will be reported elsewhere.

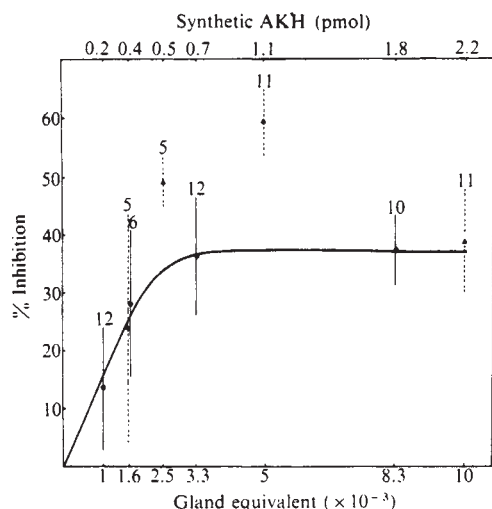


Fig. 1 The inhibition of *in vitro* protein synthesis by adult fatbodies incubated with homogenates of the glandular lobe of the CC (●), concentrations expressed in gland equivalents per 0.5 ml, and synthetic AKH (▲), concentrations expressed in pmol per 0.5 ml. Because the glandular lobes of the adult locust contain about 50 μg of AKH¹ the abscissae for synthetic hormone and for gland homogenates are approximately equivalent. To calculate percentage inhibition, incorporation of ^3H -leucine into half fatbodies incubated with gland homogenates or synthetic hormone was compared with that occurring in the contralateral half incubated with optic lobe homogenate. Bars represent twice the standard error. Integers above the bars indicate the number of replicate experiments. Synthetic AKH was a gift from Dr R. Downer.

The insects used in these experiments had a complete neuro-endocrine system, and it was possible that the response detected was the result of the activation of one or more elements of their own endocrine system. To test this possibility the fatbody was dissected out from mature adult male locusts, divided longitudinally and each half was incubated *in vitro* in 0.5 ml Schneider's modified *Drosophila* medium (GIBCO) containing 1 μCi ^3H -leucine. One half received a homogenate of one pair of glandular lobes in 10 μl of locust saline; the other received 10 μl of a similar homogenate of optic lobe in locust saline. After 45 min at 36 °C, 50 μl of the incubation medium was removed and incubation continued. After 2 h a second 50- μl sample was taken and incubation was terminated. The medium was collected; the fatbody was washed quickly in saline and then homogenised in 100 μl saline. A 20- μl sample of the homogenate was assayed for total radioactivity. A second 20- μl sample was assayed for protein¹⁴ and incorporation of radioactivity into protein¹² along with samples taken from the incubation medium. The incorporation of labelled amino acids into released protein was expressed as a function of protein in the

fatbody¹⁵. For convenience the incorporation of labelled amino acid into proteins will be termed protein synthesis although the size of the amino acid pool was not determined. Because the rate of incorporation was not immediately affected by the gland homogenate, the degree of inhibition was determined by comparing the difference in incorporation of label by the control and treated fatbodies during the last 75 min of incubation. The homogenate of 1/100 glandular lobe suppressed protein synthesis by 38% (Fig. 1). A similar level of inhibition occurred whether fatbodies were taken from fifth instar larvae (5-d-old) or mature adult males. The relationship between concentration of gland extract and the level of inhibition of protein synthesis is shown in Fig. 1. This inhibition of incorporation of label followed a similar pattern in the protein of the fatbody to that of the protein released into the incubation medium. No significant difference in the amount of label in the free amino acid pool of the control and experimental fatbodies could be detected. Gel electrophoresis¹⁶ of the medium after inhibition showed that incorporation of label into all proteins was equally affected by the gland homogenate.

Cheeseman *et al.*¹⁹ have estimated that 0.007 gland pair equivalents are released *in vivo* in response to a short flight resulting in a 135% increase in haemolymph lipid. Because the blood volume of an adult male locust is approximately 360 μl (ref. 20), Fig. 1 shows that significant inhibition of protein synthesis occurs at concentrations of AKH likely to be found in the animal.

The inhibitory activity of the gland extract was stable to boiling for 15 min and to freezing at -12 °C, although some activity was lost after both these treatments. The activity was destroyed when gland homogenates were digested with protease (Sigma) (1 mg ml⁻¹ at pH 6.5 in 0.1 M phosphate buffer for 18 h). Electrophoresis of the homogenates in 5% polyacrylamide gels showed the activity to migrate close to the ion front. These data suggested similarities between the inhibitory factor and locust AKH which is the only hormone demonstrated in the glandular lobe of the CC so far¹⁷. The relationship between concentration of synthetic AKH and suppression of protein synthesis *in vitro* by adult fatbody is shown in Fig. 1. This relationship leads us to believe that the inhibitory activity of glandular lobe homogenates is due to the presence of AKH.

Mayer and Candy¹⁸ reported that injection of CC extracts into locusts brought about mobilisation of fatbody lipids which was similar to that during flight. It is generally accepted that AKH promotes efficient flight metabolism. It is possible that the suppression of protein synthesis in the flying insect represents another example of such a metabolic switch. The inhibition of protein synthesis by AKH may be significant in circumstances other than flight. AKH is present in larval locusts in which it exerts little effect on lipid mobilisation⁵ but a considerable effect on protein metabolism. It is possible that the inhibition of protein synthesis is the predominant action of AKH in larval locusts.

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A transposon, Tn732, encoding gentamicin/tobramycin resistance

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Gentamicin and tobramycin are important antibiotics in the treatment of hospital infections because of their activity against a wide range of bacterial genera. With their increasing use, bacteria resistant to these drugs have appeared, the resistance being frequently plasmid determined¹⁻⁴. The resistance genes determine various enzymes that modify and inactivate the drugs⁵ and there is association between particular gentamicin/tobramycin resistance genes and plasmids of particular groups^{6,7}, implying that acquisition of such a gene by any plasmid is a rare event⁴. We now report the identification of a transposon or 'jumping gene' encoding the gentamicin/tobramycin adenylating enzyme, ANT(2''), on a plasmid of incompatibility group FII (IncFII).

This plasmid, pHH1313b, confers resistance to ampicillin, tetracycline, chloramphenicol, gentamicin and tobramycin (Table 1) and was identified during a study of the spread of gentamicin/tobramycin resistant *Klebsiella* in a Toronto hospital⁹. The gentamicin/tobramycin resistance is conferred by the adenylating enzyme ANT(2''). It is a non-conjugative plasmid, and to look for transposition of gentamicin/tobramycin resistance, it was mobilised into the *Escherichia coli* K12 *recA* strain, PB1455 (*thr⁻leu⁻thi⁻*) using the plasmid R751 (Table 1). Another IncFII plasmid, R1, conferring resistance to ampicillin, kanamycin, sulphonamide, chloramphenicol and streptomycin (Table 1) was transferred into PB1455 (pHH1313b.R751) by conjugation. To look for transposition of gentamicin/tobramycin resistance to R1 we selected for the transfer of gentamicin/tobramycin resistance into the K12 strain J62-2 (*pro⁻his⁻trp⁻rif^R*). All of the gentamicin/tobramycin resistant transconjugants obtained were screened for the other resistance markers of pHH1313b, R1 and R751. Ninety per cent of them carried the resistances of pHH1313b and R751 and had resulted

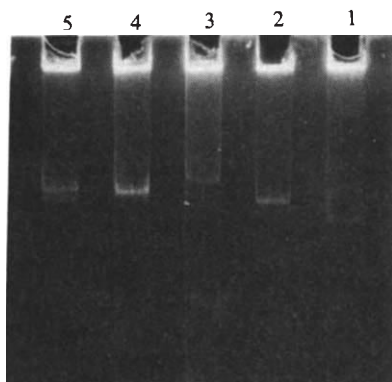


Fig. 1 Single colony lysates of R1 and R1:Tn732. Colonies were lysed directly on top of a 0.7% agarose vertical slab gel as described by Eckhardt¹⁰. Standard plasmids of known MW were included. 1, R702, 46×10^6 ; 2, R1, 62.5×10^6 ; 3, RA1-1, 85×10^6 ; 4, R1:Tn732, 70×10^6 ; 5, a colony of R1:Tn732 + R1 lysed together in the same well. Gels were run for 6 h at a constant voltage of 170 V as described in ref. 4. MWs of standard plasmids were obtained from ref. 8.

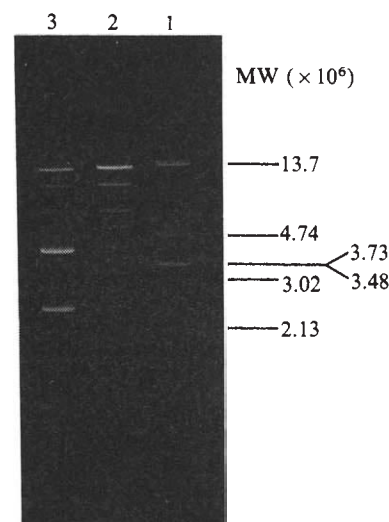


Fig. 2 *Eco*RI restriction enzyme analysis of R1 and R1:Tn732. Purified plasmid DNA was isolated by a sarkosyl-cleared lysate method (K. Hardy, personal communication). Digestions were performed as described in ref. 4. Digested DNA was run on a 0.7% agarose horizontal submerged gel of dimension $20 \times 20 \times 0.4$ cm for 3 h at a constant current of 80 mA. 1, *Eco*RI digested λ DNA; 2, *Eco*RI digested R1 DNA; 3, *Eco*RI digested R1:Tn732 DNA. Gels were calibrated using the known molecular weights of the λ *Eco*RI fragments¹² and the MWs of the R1 *Eco*RI fragments¹³.

from the mobilisation of pHH1313b into J62-2 by R751. However, 10% carried the resistances of R1 plus gentamicin/tobramycin resistance. Some of these colonies were lysed on top of 0.7% agarose vertical slab gels using the method of Eckhardt¹⁰ in order to show their plasmid content. In each case one plasmid of molecular weight (MW) 70×10^6 was seen (Fig. 1). Thus the transposition of gentamicin/tobramycin resistance to R1 resulted in an increase in MW of 7.5×10^6 . We have designated this transposed sequence Tn732. The frequency of its transposition to R1 was 1 in 10^6 which is low, compared with other transposons, although within the reported range¹¹.

Purified DNA was isolated from R1 and from one of the clones of R1:Tn732. The two plasmids were compared by digestion with the restriction enzyme *Eco*RI (Fig. 2). In this case Tn732 had inserted into R1 in such a way that the difference between R1 and R1:Tn732 is the presence of two extra fragments with MW of 4.3×10^6 and 2.8×10^6 (Fig. 2). Further analysis of *Eco*RI digests using 2% agarose gels, which showed the smallest *Eco*RI fragments of R1 (ref. 13), confirmed that the only differences between R1 and R1:Tn732 were the extra fragments in R1:Tn732. It appears that Tn732 had inserted into an existing *Eco*RI site, duplicating the site and generating the extra fragments.

This is the first report of the identification of transposable gentamicin/tobramycin resistance. Transposons increase the chances of dissemination of resistance genes and it will be interesting to know how widespread Tn732 is among bacteria

Table 1 Plasmids used in this study

Plasmid	Resistances	MW	Inc group
pHH1313b	ApTcCmGmTm	62×10^6 *	FII
R1	ApCmSmSuKm	62.5×10^6 †	FII
R751	Tp	30×10^6 †	P

Ap, ampicillin; Tc, tetracycline; Cm, chloramphenicol; Gm, gentamicin; Tm, tobramycin; Sm, streptomycin; Su, sulphonamide; Tp, trimethoprim; Km, kanamycin.

* M.E.N., unpublished observation.

† From ref. 8.

and their plasmids. Work is in progress to characterise Tn732 further using restriction enzyme analysis. Once the finer structure of Tn732 is known we hope to use a radioactively labelled fragment of Tn732 in a filter hybridisation assay to show its spread elsewhere.

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Carboxy-terminal amino acid sequence of α -tubulin from porcine brain

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Tubulin is the main protein of microtubules, which are found in all eukaryotic cells. These structures participate in cell division, intracellular transport and secretion processes, ciliary and flagellar movement, morphogenesis and cell orientation. Tubulin is generally assumed to consist of two different chains, α and β , each of molecular weight about 55,000, which form a heterodimer in solution¹. Recently, however, heterogeneity within each type of chain has been claimed on the basis of immunological non-identity², peptide mapping³, isoelectric focusing and gel electrophoresis^{4,5}. Another type of heterogeneity has been studied in more detail: a specific ligase binds one tyrosine residue to the carboxy-terminal residue of only a fraction of the tubulin α -chain⁵⁻⁷. The enzyme has been found in many tissues of various species^{8,9}, but the biological significance of the reaction is unknown. Elucidation of the primary structure should clarify whether a family of tubulins exists within one organism, and whether tubulin functions are regulated by post-translational modification. Furthermore, if a general principle underlies the various intracellular movements, one might expect sequence homologies between tubulin and muscle proteins. We have now established the sequence of the carboxy-terminal 78 amino acid residues in α -tubulin from porcine brain by Edman degradation of overlapping cyanogen bromide, tryptic and chymotryptic fragments. A terminal tyrosine was the only evidence for heterogeneity. The region is unusually acidic and not homologous to known proteins. Prediction of the secondary structure suggests a 'jack-in-the-box-like' structural change, dependent on the microenvironment.

We have purified tubulin from porcine brain by a modification of the methods used by Eipper¹⁰ and by Ludueña *et al.*¹. The 100,000g brain supernatant in 0.05 M sodium pyrophosphate buffer, pH 7.0, was incubated with 0.1 mM colchicine for 15 min at 37°C before chromatography on DEAE-cellulose with a linear gradient of 0.1-0.3 M sodium chloride. Tubulin was identified by the fluorescence of its complex with colchicine¹¹. The preparation was assayed for protein impurities by disc gel electrophoresis in the system of Yang and Criddle¹² using 8%

gels. The gels were stained with Coomassie blue and scanned in a Vernon scanner. Only tubulin of more than 95% purity was processed further.

For separation of α - and β -chains the protein was reduced and alkylated with iodoacetic acid¹³ and chromatographed on hydroxylapatite in 0.1% SDS with a linear gradient of 0.2-0.4 M sodium phosphate¹⁴. Fractions were assayed for purity by gel electrophoresis as above. Only α -chain of at least 95% purity was used for sequence determination.

The sequence of 78 residues at the carboxy terminus of tubulin α -chain is shown in Fig. 1. It must represent the terminal region because it contains the only tryptic peptide without a basic residue and the only cyanogen bromide piece devoid of a methionine-derived homoserine lactone. Furthermore, the terminal Glu corresponds to the glutamic acid previously determined to be the last amino acid in the α -chain¹⁷.

The entire segment is devoid of proline and glutamine and has an acidic 'tail'. Residues 78-41 have a net charge of +4, are rich in alanine and in large side chains, whereas the last 40 positions have a net charge of -17, and are extremely rich in glutamic acid (40%) and glycine. It is by far the most acidic region of the α -chain and one of the most acidic sequences known.

Some heterogeneity could be expected from isoelectric focusing data. In this region of the chain, however, we found only one such case: the terminal tryptic peptide was isolated in two forms. About 85% of the material has nine Glu and one Tyr residues (composition Asp 1.88, Ser 1.08, Glu 8.80, Gly 3.99, Val 3.18, Tyr 1.07), whereas 15% shows nine Glu and two Tyr residues (Asp 2.03, Ser 1.17, Glu 8.89, Gly 4.09, Val 3.21, Tyr 1.60). The additional tyrosine is located in the C-terminal position. This corresponds to the observed post-translational binding of a tyrosine residue to the terminal glutamic acid by a tubulin-specific ligase⁶. Only about 45% of α -chain molecules can be tyrosylated⁸. This may be due to the presence of two different α -chains, although the C-terminal part has a homogeneous sequence. A cyanogen bromide fragment, comprising 26 residues almost identical to our C-terminal CNBr peptide, was isolated by Lu and Elzinga¹⁸ from calf brain α -tubulin. These authors found one more glutamic acid at the C-terminus and discuss the possibility of a heterogeneity of Gly-Glu-Glu-Glu or Gly-Glu-Glu-Tyr. In our source, amino acid analyses of the two forms of tryptic peptide clearly show that tyrosine is added to a peptide of otherwise identical composition. Two other peptides,

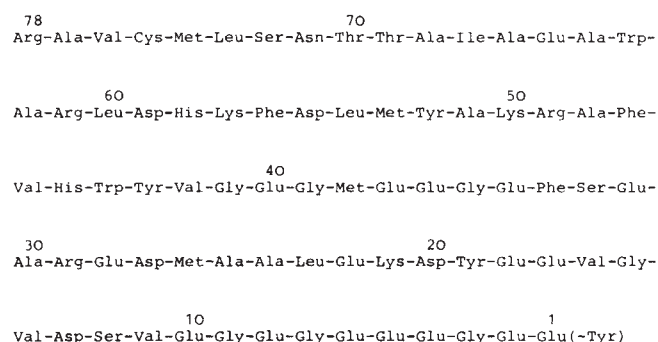


Fig. 1 Amino acid sequence of the C-terminal region in α -tubulin. Residues are numbered starting at the carboxyl terminus. Batches of about 200 mg of α -chain were cleaved with trypsin, chymotrypsin and cyanogen bromide, respectively. Enzymatic digests were fractionated on Dowex 1X2 in volatile buffers and re-chromatographed on phosphocellulose or cellulose thin layer. Larger enzymatic fragments and the cyanogen bromide cleavage products were separated on columns of Sephadex G-50 Superfine (190×1.5 cm) in 8 M urea containing 0.1 M ammonium bicarbonate, and desalted on Sephadex G-10. Amino acid analyses, enzymatic digestions and sequence analyses by automated and manual methods were carried out as described previously¹⁵, except that HPLC was used instead of gas chromatography for the identification of degraded phenylthiohydantoins¹⁶ and assignment of side chain amides.

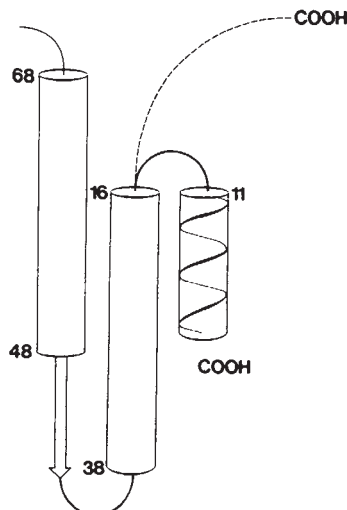


Fig. 2 Predicted secondary structure of the C-terminal region in α -tubulin. Residues 68–48, 38–16 and 11–1 are helical, position 47–42 represents a strand of a β -sheet and residues 41–38 and 15–12 are reverse turns. A jack-in-the-box transition of the labile terminal α -helix to a more extended structure is proposed to take place. The stability of each configuration depends on the microenvironment because of the many glutamic acid side chains, which may repel each other if fully dissociated. A C-terminal tyrosine increases the lability of an α -helix.

characterised but not ordered by Lu and Elzinga¹⁸, match residues 62–53 and 37–26 in our sequence.

We could not detect any significant homologies to other known protein sequences, including bacterial flagellin, nucleotide and calcium binding proteins, actin and other muscle proteins.

The procedure of Chou and Fasman¹⁹ was used to predict the secondary structure of this region (Fig. 2). It yields an α -helix at residues 68–48 (helix potential $P_\alpha = 1.20$ compared with potential for a β -sheet $P_\beta = 0.96$), a strand of a β -sheet at residues 47–42 ($P_\alpha = 1.00$, $P_\beta = 1.42$) which is connected by a reverse turn at positions 41–38 (turn potential $P_t = 1.12$, $P_\alpha = 1.03$, $P_\beta = 0.73$) to a second helix at positions 38–16 ($P_\alpha = 1.26$, $P_\beta = 0.69$) followed by another reverse turn at positions 15–12 ($P_t = 1.24$, $P_\alpha = 0.85$, $P_\beta = 0.93$). This clearcut situation becomes increasingly labile towards the C-terminus. Residues 11–1 have a helix potential ($P_\alpha = 1.21$, $P_\beta = 0.59$), but the many carboxyl groups of the glutamic acid residues, if fully dissociated, may repel each other, as polyglutamic acid loses its helical conformation at about pH 5.5 and turns into a random coil at higher pH²⁰. Furthermore, the interspersed glycines create an underlying potential for turns and the helix potential is decreased by the addition of tyrosine.

We propose a jack-in-the-box-like structural change of the C-terminal residues from a helix to a more extended structure, depending on the microenvironment, for example, a tubulin dimer in solution or incorporated in a microtubule. Experimental support for two different states of the C-terminus of the α -chain comes from the finding by Thompson *et al.*²¹ that the post-translationally added tyrosine is exchanged much more rapidly in intact microtubules than in the soluble dimer.

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Stomatal conductance correlates with photosynthetic capacity

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Previous studies on the physiology of stomata in higher plants suggest that stomata influence the rate of CO₂ fixation in leaf mesophyll tissue. We believe that an equally important stomatal function has not been fully recognised; that stomatal aperture is determined by the capacity of the mesophyll tissue to fix carbon. We altered the capacity of leaves to fix carbon by various means, and found invariably that the diffusive conductance of the epidermis to CO₂ transfer, g , (which mainly depends on the number and dimensions of the stomata) changes in nearly the same proportion as the rate of assimilation of CO₂. Thus, the intercellular concentration of CO₂ (c_i), calculated as $c_i = c_a - A/g$ (where c_a is ambient concentration of CO₂, A is assimilation rate of CO₂), tends to remain constant providing c_a is kept constant. We used routine techniques¹ to measure A and estimate g in leaves placed singly in chambers. Conductance takes account of CO₂ transfer through both stomata and leaf boundary layer, the conductance of the latter being $0.5 \text{ mol m}^{-2} \text{ s}^{-1}$.

Figure 1 shows the effects of applications of 3-(3,4-dichlorophenyl)1,1-dimethylurea (DCMU), an inhibitor of photosynthetic electron transport, and abscisic acid (ABA), a hormone which decreases stomatal aperture, on assimilation rate and leaf conductance in cotton plants. When DCMU was applied, A and g declined in the same proportion, the slope of the line implying that c_i is constant at $220 \mu\text{l l}^{-1}$. When ABA was applied, a curvilinear relationship between A and g was obtained. Its shape is consistent with the supposition that A declined as a result of decrease in intercellular CO₂ concentration brought about by partial closure of the stomata. A similar contrast is shown in Fig. 2. The four points lying almost on a straight line represent A and g in *Eucalyptus pauciflora* at four different irradiances. The line does not extend through the origin and thus c_i was not quite constant in this instance. However, the change was only slight; c_i decreased from 257 to $243 \mu\text{l l}^{-1}$ with increasing irradiance. The curves in Fig. 2 were computed from measurements of the influence of c_i on A (c_i having been varied by varying c_a) and use of the equation above with c_a taken as $320 \mu\text{l l}^{-1}$. They are estimates of the relationships between A and g that would be manifest at each irradiance if g were directly perturbed, as was done in the cotton plants by use of ABA. Figure 3 shows rate of assimilation and leaf conductance in plants of *Zea mays*. Differences between plants were due to differing levels of nitrogen nutrition, phosphorus nutrition and water stress. The points approximate a straight line passing through the origin the slope of which corresponds to $c_i = 100 \mu\text{l l}^{-1}$. The continuous curves show how a direct perturbation of g would have influenced A in four particular plants having differing levels of nitrogen nutrition; they were computed in the same manner as those in Fig. 2.

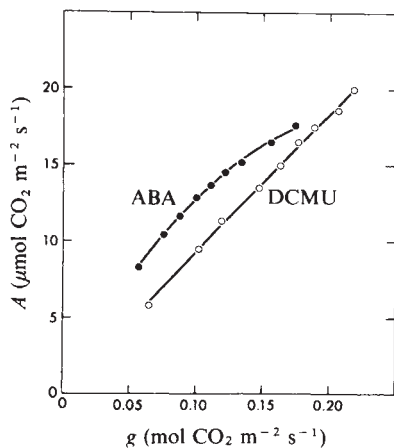


Fig. 1 Relationship between A and g in *Gossypium hirsutum* as influenced by ABA, and DCMU. ABA (1×10^{-6} M) and DCMU (5×10^{-6} M) were applied in the water taken up by the detached, transpiring leaves. The different points represent differing periods of accumulation. Ambient CO_2 concentration, $320 \mu\text{l l}^{-1}$, leaf temperature, 30°C ; leaf to air vapour pressure difference, 20 mbar.

The treatments which yield the straight line relationships in Figs 1–3 all influence the mechanisms of photosynthesis at the cellular level. The inhibitor DCMU reduces photosynthetic electron transport; increase in irradiance increases the potential rate of electron transport; nitrogen nutrition influences the amounts of chlorophyll and the activities of ribulose 1-5-bisphosphate (RuBP) carboxylase and phosphoenol pyruvate (PEP) carboxylase; and phosphorus availability affects photophosphorylation and the development of chloroplast lamellae². Also, water stress, imposed slowly, caused a decline in both A and g such that intercellular CO_2 concentration remained essentially constant (Fig. 3), the implication being that stress directly affects photosynthetic carbon fixation. Diminution in the amount of soil water resulted in a 40% decrease in A and g but c_i remained constant at $100 \mu\text{l l}^{-1}$. There are many other reports of observations of gas exchange which demonstrate the same proportional relationship between g and A ; for example in *Phaseolus vulgaris*^{3,4} and *Oryza sativa* leaves of various ages⁵, in *P. vulgaris* and *Zea mays* plants grown at different irradiances during ontogeny⁶, in *O. sativa* plants given different amounts of nitrogen⁷, in leaves of *P. vulgaris*, *Z. mays* and *Imperata*

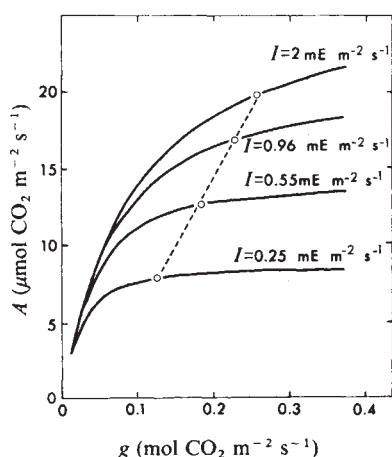


Fig. 2 Relationship between A and g in *Eucalyptus pauciflora*. A and g at four irradiances, I , during measurement with ambient CO_2 concentration = $320 \mu\text{l l}^{-1}$, leaf temperature = 25°C , leaf to air vapour pressure difference = 18 mbar. Curves represent the variation of A that would occur if g were independently perturbed; they are computed from measurements of the responses of A to changes in intercellular CO_2 concentration.

cylindrica subjected to different irradiances⁴, and in *Z. mays*⁸, *Sorghum bicolor*⁹ and *Eucalyptus socialis* seedlings¹⁰ subjected to increasing water stress.

In eight C_3 plant species c_i was maintained at about $220 \mu\text{l l}^{-1}$ and in four C_4 species it was maintained at about $100 \mu\text{l l}^{-1}$. These observations were made at an ambient CO_2 concentration of $320 \mu\text{l l}^{-1}$, with a vapour pressure deficit of 20 mbar and with leaf temperatures near the optima for photosynthesis. The influence of leaf temperature on the relationship between g and A differed from that of the other factors so far considered. In general it was such that c_i increased at both low and high temperatures.

Linear relationships between A and g do not necessarily indicate that stomata control the rate of assimilation. When the source of variation was a treatment which directly affected stomata only, as with the application of ABA¹¹, the resultant variation in rate of assimilation (caused by the influence of conductance on c_i) was different (Fig. 1). Such curves also occur as a result of variation in ambient humidity^{12,13} and rapid imposition of water stress^{14,15}.

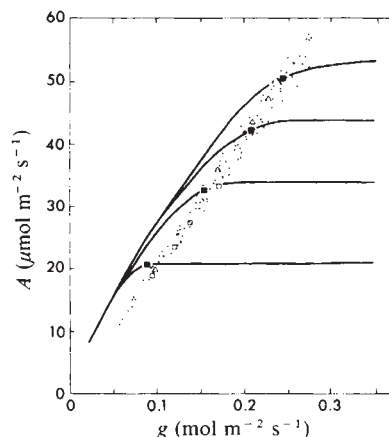


Fig. 3 Relationships between A and g in *Zea mays*, measured at irradiance of $2 \text{ mE m}^{-2} \text{ s}^{-1}$, ambient CO_2 concentration, $320 \mu\text{l l}^{-1}$, leaf temperature, 30°C and leaf-air vapour pressure difference, 20 mbar: $\bullet$, plants having received different amounts of nitrogen and phosphorus nutrients; $\square$, different stages in the recovery of a nitrogen deficient plant after nitrogen nutrient was given; $\triangle$, different stages in the development of water stress in a plant from which water was withheld. The continuous curves represent the variations in A that would occur in four particular plants having differing photosynthesis capacities due to differing levels of nitrogen nutrient if g were independently perturbed. $\blacksquare$ Indicates actual values of A and g corresponding to c_a of $320 \mu\text{l l}^{-1}$.

What, then, are the mechanisms responsible for the linear relationships in Figs 1–3? It is possible that stomata respond directly and independently to factors that influence photosynthetic carbon fixation. It is known that DCMU^{16,17}, irradiance^{16,17} and water potential¹⁶ influence stomatal aperture in isolated epidermal strips. Perhaps nitrogen and phosphorus also do so. Nevertheless, we think that the close correlation between A and g in a variety of conditions, including those associated with development suggests that there is a direct link between the two. The discovery¹⁸ that the abaxial and adaxial patterns of stomatal response to light in a periclinal chimaera—between *Solanum pennellii* epidermis and *Lycopersicon esculentum* mesophyll—were almost identical to those in *L. esculentum*, and quite unlike those in *S. pennellii*, supports this notion.

A likely hypothesis is that stomata respond to changes in c_i so as to maintain it at a constant level by negative feedback. Raschke¹⁶ and Raschke *et al.*¹⁹ have attributed the variation in conductance with variation in irradiance to this mechanism. If the feedback loop maintains c_i constant at a preset value when irradiance is changed, then c_i should also remain constant when ambient CO_2 concentration is changed. Figure 4 illustrates a test of this last proposition. It shows that $\partial c_i / \partial c_a$ is about 0.7 in

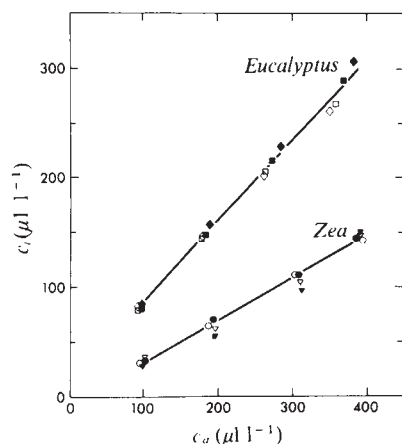


Fig. 4 Relationship of c_i to c_a in *Eucalyptus pauciflora* at four irradiances during measurement: $\blacklozenge$, $0.25 \text{ mE m}^{-2} \text{ s}^{-1}$; $\blacksquare$, $0.55 \text{ mE m}^{-2} \text{ s}^{-1}$; $\square$, $0.96 \text{ mE m}^{-2} \text{ s}^{-1}$; $\blacktriangle$, $2.0 \text{ mE m}^{-2} \text{ s}^{-1}$; and in *Zea mays* grown at four nitrogen levels. The *E. pauciflora* plant is that used to obtain the data shown in Fig. 2, and the *Z. mays* plants are those used to obtain the four continuous curves in Fig. 3.

Eucalyptus pauciflora and 0.4 in *Zea mays*. Apart from a report⁴ concerning *Zea mays* at high c_a , we know of no instance when the coefficient has been found to be near zero¹¹. Therefore negative feedback cannot entirely account for the near constancy of c_i when A is perturbed¹.

We consider that our evidence suggests an additional mechanism: that the stomata respond to another metabolite of photosynthesis in the leaf mesophyll tissue. There are several metabolites (such as ATP, NADPH and ribulose biphosphate), the concentrations of which partially determine the capacity of the photosynthetic system to fix carbon, but decrease with an increase in the rate of carboxylation associated with an increase

in intercellular CO_2 concentration. Their concentrations vary in a complex way with temperature. Our observations would be explained if stomatal aperture were related to the concentration of one of these compounds. This would be consistent with the tendency for stomata to close when the ambient concentration of CO_2 is increased. The suggestion also agrees with evidence of metabolite transport between mesophyll tissue and guard cells²⁰.

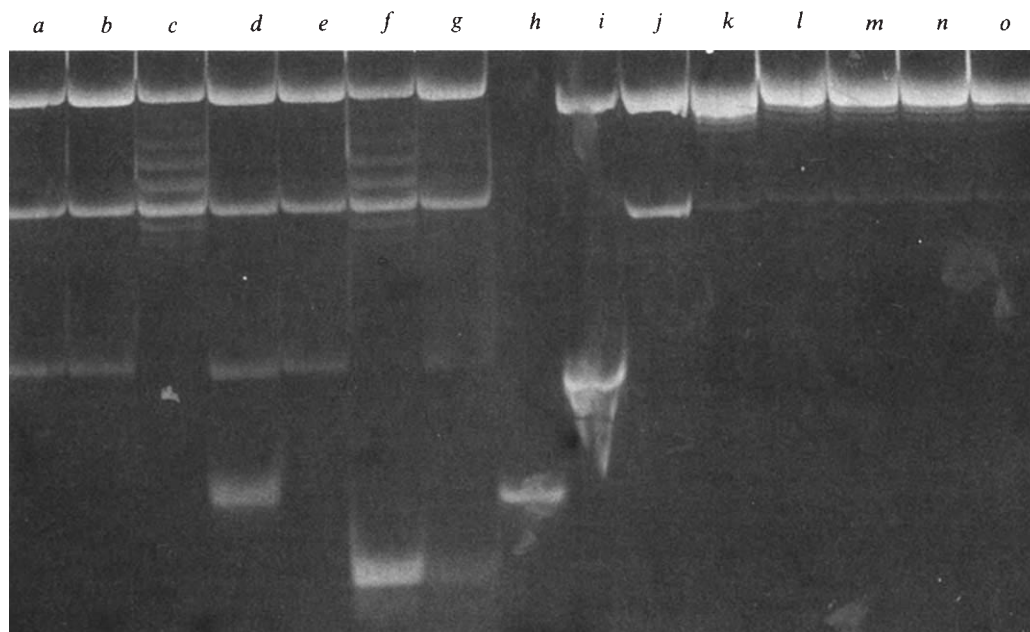
The extent to which stomatal responses modulate the rate of transpiration has been emphasised in the literature. However, it is clear that stomatal aperture is a compromise between the needs to conserve water and to maintain the rate of assimilation at a level dependent on the intrinsic capacity of the leaf mesophyll tissue to fix carbon. Our evidence indicates how closely conductance is influenced by that capacity and is relevant to theories of optimality in the regulation of gas exchange²¹⁻²³.

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Erratum

In the article 'Single strands induce recA protein to unwind duplex DNA for homologous pairing' by R. P. Cunningham *et al.* *Nature* **281**, 191-195, Fig 4 was reproduced such that some of the bands were lost. It is shown in a different form below.



reviews

Adapt or be damned

Eric Ashby

Uncertain Futures: Challenges for Decision-Makers. By R.U. Ayres. Pp.429. (Wiley-Interscience: New York and Chichester, UK, 1979.) £13.

To call futures 'uncertain' is an understatement. All that can be said with certainty about the future is that publishers still welcome books about it. Good luck to the publishers, for these books keep the debate going, and that is important. Dr Ayres makes useful contributions to this debate, though in style and content the book is in places exasperating. His contributions to the debate are that he gives priority to social and political issues and that he writes from practical experience as an industrial consultant whose living depends on the common sense of the judgements he makes; so his essay is comparatively free from the irresponsibilities of academic utopians and doomsters.

The preface is excellent. To forecast the future, Dr Ayres says, one has to examine not only technological issues, "but the trends in the culture — in society's political and social life, in . . . its people's attitudes, beliefs, and customs". Time after time, "it turns out to be social, political, ideological, legal, economic, and religious issues that stand in the way of technological solutions to human problems". He makes the distinction between projections — contingent statements which are valid provided all the 'if' clauses are clear — and predictions of what *will* happen in the future, most of which, he says, "are either disreputable or ambiguous". And, as Dr Ayres' examples show, most projections, though valid, are so weakened by the 'ifs' that they are precious little use to decision-makers. He backs up his argument with nice examples: thus IBM and Kodak lacked the foresight to invest in the infant technology of xerography, and a distinguished astronomer royal is reported to have said, one year before Sputnik I went up, that "space travel is utter bilge".

Dr Ayres' approach to the practice of forecasting is sober and systematic. First, the forecaster himself must be scrutinised. Is he a conservative who sees change as incremental projections from the present? Or a radical who sees change as cataclysmic discontinuities with the present? Does he create scenarios using as a tool a computer or his imagination? Is his approach clinical and objective or does he speak as a

committed advocate? What is his implicit theory of history? Does he believe that leaders really lead? Or do they simply articulate what 'society' believes? He makes the important point to which mathematicians are now trying to apply catastrophe theory: namely that human institutions do not adjust to small pressures. The pressures build up — as the earth-stresses do before an earthquake — until a critical point is reached and there is an explosion; it happened in Lebanon, in Ulster, in the Southern States of America, in Rhodesia; and it is likely to happen over the energy shortage in the United States and the food shortage in India. (There is a wry similarity between the gas-guzzling Cadillac in America and the 100 million or so sacred cows in India: the one wasting oil resources, the other wasting food resources; the one a symbol of the religion of materialism, the other a symbol of an ancient oriental religion.)

After this admirable introduction, the book becomes disappointing. Dr Ayres makes a systematic appraisal of the present state of Mankind (he calls it 'measures of man'); man's values, his international relations, his prospects judged from the present levels of population and economics, his technology and the way it seems to be going, and the resources left in the world (and from the Sun) to carry Mankind into the next millennium. He uses familiar examples and draws on secondary sources (and some of them not very expert sources at that). To judge him by his own criteria for scrutiny, he is an incrementalist with misgivings about the prospects for incrementalism; a forecaster who prefers to use imagination rather than to rely on quantitative data; an analyst who tries hard to follow his own recipe (forecasters must be "indifferent to outcomes") but whose personal convictions keep breaking in. The bulk of the book records Dr Ayres' views on these 'measures of man' supported by telling anecdotes and some rather sketchy data. This is no bad thing; there are plenty of data to be found elsewhere, but the book has to be judged on the originality of its reflections rather than the comprehensiveness of its information.

I think it would be a fair summary of Dr Ayres' views to say that they are an articulation of what in Britain might be called sound CBI (Confederation of British Industry) doctrine. He has the courage to say some unpalatable things. He is apprehensive about the future of liberal

democracies and he holds out little hope that Britain's brand of 'soft socialism' which stresses equity and freedom at the cost of efficiency, will even be able to preserve equity and freedom in the long run. He makes a list of wealth-creating values (motivation, positive attitude to work, integrity and personal honour, concern about the future) and describes how these are being eroded. (He might have added loyalty to the firm, to the institution, to the employer, for the erosion of these loyalties is the most obvious national danger.) He has some robust down-to-earth things to say about some of the current panaceas we talk about. Plans for rural development, for example, he thinks are impracticable, for the infrastructure necessary must be created by an urbanised society; and, with the prospect that transport will become more difficult as the oil shortage bites, there may be a revival of urban living. Among the attitudes which contribute to the growing malaise in western civilisation Dr Ayres selects two for special treatment: one is the resistance to innovation exemplified by a wide variety of practices, from the 'catastrophic' regulations which inhibit the production of new pharmaceutical drugs, to the attitude of some trade unions which (as he bluntly puts it) is to "increase the number of man-hours required to produce a unit of output, *at no decrease in wages*, as a device to create even more unnecessary jobs". The other attitude which Ayres criticises is the proliferation of what he calls "parasitic professions". Lawyers, in particular, get his goat: "the most parasitic single group in the United States . . . a unique ability to create demand for their own unproductive services". Health services, battenning on Blue Cross, come in for similar, though milder, criticism. He challenges the medical 'ethics' which prevents doctors from competing by offering definite services for fixed prices, and he thinks fewer resources ought to be spent on the unproductive practice of keeping old people alive who suffer from degenerative diseases. Education is another social activity which, in Dr Ayres' opinion, is failing to equip the next generation to cope with its uncertain future. There is, he believes, a decline in quality of education due in part to unionisation among teachers ("the organization of effective teachers' unions has tended to reduce the average level of performance by the teachers . . ."), in part to time wasted looking at TV, and in part due to the use by children

of minicalculators.

When Dr Ayres talks about technology he is on firm ground, but his knowledge of economics and political science unfortunately does not match his admirable determination to give these subjects a higher priority than technology as determinants of the future. However, towards the end of the book, in the last 75 pages, he no longer comments on familiar facts; instead he gives free rein to his imagination. He describes, in the form of fictitious brief press extracts, the way in which a world energy crisis might develop in the west, or a food crisis in the Third World. As a scenario for the possible political response to an oil shortage between 1981 and 1985, I found the 18 pages of 'press cuttings' far more convincing than any sophisticated computer print-out. Ayres cites precedents from past history for some of his speculations about future history, and he presents the reader with a spine-chilling but quite credible series of social reactions which might follow another embargo of oil from the Middle East.

Forecasters, writes Dr Ayres in his preface, "must be independent-minded and indifferent to outcomes". He may have started to write this book as a true-blue forecaster. He ends the book as an advocate. Perhaps that is the moral to be

got from the book: that the intervening chapters dispel indifference. It is as well they should, for what emerges from this book, despite its superficialities and its flimsy supporting evidence, is not superficial at all. It is that democratic institutions, "never deeply rooted in most countries, have already proved to be pretty much incapable of grappling with problems for which all the solutions are painful". This, in my view, is a more imminent and serious danger than the danger of nuclear war. And an insidious danger, too, for there is no external enemy to be defeated: the enemy is within and invisible. President Carter asked the American people to regard the energy crisis as the "moral equivalent of war". But this is to ask a nation to adapt itself *in anticipation* of an environmental change, not — as happens with organisms other than man — as a *response* to the change after it has occurred. The American people have not responded. The sixty four dollar question is: will communities of people respond in anticipation in a way that communities of animals would not? We may not have to wait long now for an answer. □

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Perceptual phenomena

Handbook of Sensory Physiology. Vol. VIII: *Perception*. Edited by R. Held, H. W. Leibowitz and H. L. Teuber. (Springer: Berlin, Heidelberg and New York, 1978.) DM240; \$120.

OUR knowledge of perception has advanced in bursts. The mid-nineteenth century saw the inauguration of psychophysics, the doctrine of specific nerve energies, the foundation of colour vision, the discovery of the horopter, and the remarkable insights of Helmholtz. Between 1912 and the early 1930s, the discoveries of the Gestalt psychologists posed a whole new set of problems.

After a period of comparative quiescence, the modern era began in the mid-1950s. Since then, thanks to the development of many new techniques and the ingenious refinement of old ones, a wealth of new phenomena has been uncovered. The use of microelectrodes to record from single cells has revealed much about the amazingly systematic wiring that underlies the early stages of visual processing, and has made it possible to explain at least some psychophysical results in terms of known neurophysiological mechanisms. Thus, the dispute between Helmholtz and Hering

about whether colour vision was achieved by a three colour system or by two pairs of opponent colours has been resolved: in the retina there are three different cone pigments, but more centrally hue is recoded into two mechanisms each based on opponent colours. Many other psychophysical phenomena, for example, those connected with visual acuity, stereoscopic vision and the perception of motion, also fit in with recent physiological findings, though their detailed interpretation is often still in dispute. We are, for example, still uncertain of the exact physiological correlates of the after-effects of prolonged inspection of motion or of a tilted line: some argue for a passive process of neuronal adaptation, others for a more active inhibitory mechanism.

Moreover, much recent work has served only to emphasise the extreme complexity and subtlety of perceptual mechanisms without necessarily clarifying them. In speech perception we have little idea of how phonemes are extracted, though we do know that there are no separate segments of the speech sound wave that correspond in a one-to-one fashion with individual phonemes. Despite the mass of work undertaken using new physiological and psychological techniques to investigate perception in infants and young animals, we do not understand the subtle interplay between maturation and experience. Nor is there any secure explanation of visual masking — the obliteration of one stimulus by a second presented several milliseconds

later.

Perhaps even more dispiriting is the fact that many perceptual phenomena discovered long ago still await explanation: our understanding of the traditional visual illusions is no more secure today than at the end of the last century; nor do we know any more about the mechanisms that enable us to see an object as of the size it really is, despite the variations in the retinal size caused by changes in distance.

Only a man with the bravery and erudition of Hans-Lukas Teuber could have undertaken the task of editing a volume reviewing all the disparate results and theories that have emerged in recent years. He died before he had finished the job and it was left to the two co-editors whom he had co-opted to complete it. The finished product is worthy of his erudition. I can vividly recall him as he stood confronting one of his graduate students and saying in consternation, with that characteristic raise of his bushy eyebrows, "You mean you've never *heard* of Imaizikie?". The publication of Volume VIII of the *Handbook of Sensory Physiology* removes the wretched graduate student's last excuse for such ignorance. It provides a comprehensive source of references on the topics covered, but I feel it would not have met Luke Teuber's exacting standards in other ways. With the exception of his own contribution, the writing is for the most part pedestrian and fails to convey any sense of enthusiasm or excitement. Too many of the authors concentrate on listing facts or on attempting to resolve the many disagreements between recent results: they fail to place the findings they record in the context of what perception is all about. There are, of course, exceptions such as A. M. Liberman and M. Studdert-Kennedy who write on phonetic perception.

The vast number of phenomena recorded in this *Handbook* would have been easier to assimilate had more of the authors asked themselves what light the findings throw on the way in which perceptual tasks are carried out. Indeed, the one approach to the subject that is almost wholly missing from this volume is the theoretical study of precisely this question, that is, work on computational models of perception. Such research has revealed the role of the mechanisms discovered by microelectrode work: they provide a description of the retinal image that can in turn be manipulated to yield a representation of the external world.

In short, this *Handbook*, despite the solemnity of its approach, fails to make much sense of perception, and the boring parade of findings it presents is not worth the outrageous price asked by the publishers.

Stuart Sutherland

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Behavioural neurobiology handbook

Handbook of Behavioral Neurobiology. Vol.2: *Neuropsychology*. Pp.566. Edited by M.S. Gazzaniga. (Plenum: New York, 1979.) £35.

The general public expect from academic psychologists useful, and preferably unexpected and striking, insights into the human condition. They are interested in the psychology of conscious experience rather than in the minutiae of behaviour and performance usually discussed in academic lecture theatres. It is arguable that while they remain concerned only with human performance academic psychologists can seldom be interesting and never surprising. At best they can clarify the precise nature of limitations of which we have all been aware all our lives, and they make their greatest contribution only by suggesting models for central nervous system function which explain why we have these particular limitations and not others.

In contrast neuropsychologists, who study changes in function after brain damage or central nervous system disease, have far more surprising and vivid stories to tell. Their recent discoveries concern the very texture of daily experience and sometimes offer scary glimpses into the darkness within. Their work raises questions about ourselves and our experience which could hardly have occurred to us in any other way. Until Weiskrantz's recent demonstrations it would have appeared merely a far-fetched philosophical exercise, based on an elementary semantic misconception, to enquire whether any alert and articulate human being could, deliberately on request, make accurate discriminations between patterns which he cannot *consciously* report seeing. It is also hardly likely that we should have asked, as Le Doux, Wilson and Gazzaniga do in this book, not only whether the two hemispheres of the brain have different and independent functions but also whether, when separated surgically, they may have some degree of independent conscious experience while they share, *but independently interpret in different ways*, a commonly experienced shift in emotional tone controlled by lower brain centres communicating with both independently.

This second volume of the '*Handbook of Behavioral Neurobiology*', edited by Gazzaniga, provokes many such startling questions. It also illustrates many of the conceptual and practical difficulties which beset this fascinating field of new knowledge about ourselves.

The title *Handbook* raises expectations of a detailed comprehensive reference work. Few of the contributors seem willing to attempt this useful task. Exceptions are the chapter on aphasia by Hecaen, on the frontal lobes by Jonander and Gazzaniga, on thalamic mechanisms in language by Brown, and a useful chapter on long term consequences of central lesions by Newcombe and Ratcliff.

Only a concise chapter on assessment of cognitive deficits in brain-injured patients by Goodglass and Kaplan literally meets the promise of the book's title by providing a handy guide to a complex applied literature for novice practitioners. This chapter, inadvertently, highlights a crucial difficulty currently obstructing progress in neuropsychology. Most tests of function currently used by neuropsychologists and neurosurgeons are crude, pragmatic survivals from wards and consulting rooms fifty or a hundred years ago. They have survived because they are rapidly administered indices which have been found, by experience, to very roughly categorise particular kinds of deficit when and if they are supplemented by the (often brilliant) intuitions of clinicians who, ritualistically, still administer them. A human experimental psychologist, accustomed to constructing models for normal performance at similar tasks, is at once struck by the fact that these particular tests involve such a complex muddle of interacting performance indices that it is quite impossible to say what are the precise functional deficits in performance any of them can demonstrate. However well they may serve as *ad hoc* diagnostic tools (not too well, reading between the lines of Goodglass and Kaplan's lucid chapter) they as yet give us little insight into the fascinating changes in function which actually accompany particular brain lesions.

The obvious solution is for human, experimental psychologist, knowledgeable about specific indices of performance, to interest themselves in the problem of defining precisely how performance changes when brain lesions occur. Morris Moscovitch's chapter 17 ('Information Processing and the Cerebral Hemispheres') is the nearest attempt to this desirable end. As we see from the bulk of this useful book even crude and conceptually muddled tests of residual function do work in practical diagnosis. The crucial question is why they work, when they work (that is precisely what are the deficits which each of them actually exposes), and whether better, more clearly understood and more efficient diagnostic procedures cannot be developed from the considerable expertise built up by human experimental psychologists. From this point of view Moscovitch's chapter is disappointing. He describes only his own work. He is not concerned with questions of diagnosis but with the relationship of abstract models of human information

processing to differences in hemisphere function. This would be interesting if the experimental techniques he uses gave the results which he supposes that they do. In particular the results illustrated in Fig. 1b and Fig. 2 on pages 386-388 and Fig. 5a and b on page 389 may have a number of different interpretations other than those which he places upon them.

For me this is a very exciting book indeed. If the excitement is flawed by impatience I am sorry. But the limitations to further progress are very distressing to anyone who avidly reads chapters 1-4, 7, 9-12, 14, 15 and most particularly 17 for the *ideas*. This is a book which is a key to our current conceptualisations of what we are, and to how the thin bright film of our experience covers the complex darkness within us, out of range of our introspection but perhaps within the grasp of clever and accurate experiments. The lack of experiments is galling, but I would wish the pleasure I have had reading this book to be as widely shared as possible by other scientists of many different educations and fields.

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Permutation symmetry in chemical and physical problems

Molecular Symmetry and Spectroscopy. By P. R. Bunker. Pp. 424. (Academic: New York, 1979.) \$33.

THIS volume is the first comprehensive treatment of the use of permutation symmetry in molecules, that is, in chemical and physical problems. As such, it will be widely welcomed, and fills a void which has long plagued those who had a need for this material. Fortunately, the writing in this volume is extremely lucid.

The volume begins with a clear definition of permutation operations and groups, then adds the (space) inversion operation to lead to the complete nuclear permutation inversion group (CNPI). The author next leads the reader through the more conventional application of group theory to molecules, with, however, a quite unconventional set of emphases. This reviewer feels that persons previously unfamiliar with chemical applications of group theory would have considerable difficulty with the treatment given. Fortunately, however, one may assume today (and it seems that the author has done so, although he claims the contrary) that anyone interested in the subject matter of this book has previously attained a working knowledge of molecular point groups and their use in spectroscopic problems. The emphasis in the appropriate chapters of this volume lies with the symmetry properties of the Hamiltonian and of its eigenfunctions, a much more fundamental approach than that com-

monly pursued. After these preliminaries are dealt with in 8 chapters the real meat of the volume follows: the definition of the molecular symmetry group on the basis of permutation and space inversion and its applications, primarily to problems of molecular spectroscopy and to non-rigid molecules.

It is to be welcomed that the author has been very careful and explicit in his definitions. Thus, the emphasis on the distinction between space and molecule fixed coordinate systems has led to a clean and unambiguous distinction of the two inversion operations, E^* and i , which have been far from clear in much of the previous literature. Similarly the very careful phrasing of the conditions of feasibility, although they may at times seem redundant, make a significant contribution to the understanding of the subject matter.

The book is extensively illustrated with well-drawn figures, essential when discussing symmetry operations and their effect. The author has attempted, and it seems successfully, to illustrate each theoretical concept he introduces immediately with appropriate examples. For further emphasis, throughout the volume he has provided problems to illustrate the principles, and then given detailed solutions.

Although this book addresses itself to a relatively restricted audience — spectroscopists, and quantum mechanicians interested in classification of excited states — it may be expected to rapidly become an essential tool to this audience. It is further to be hoped that the volume will help to make more scientists aware of permutation symmetry and thereby provide new insights into other phenomena.

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Electron microscopy in chemical investigations

The Chemical Applications of Transmission Electron Microscopy. By J. R. Fryer. Pp. 286. (Academic: London and New York, 1979.) £18; \$38.

THE electron microscope finds application in almost all branches of science and technology. Over the years there have appeared many books describing its uses in biology, medicine, metallurgy and materials science, but we have had to wait until now for a treatment of its role in

chemical research. The reasons for such a lack are not far to seek. Chemistry is rarely as interested in morphology (except in polymers) as are biology and metallurgy for differing purposes. Its problems so often involve knowledge at the atomic and molecular level, whether of structure or function. In recent years, however, the instrument has found increasing application in surface chemistry (catalysts and thin films), crystal chemistry (defects, non-stoichiometry and phase changes), solution chemistry (clay minerals, polymers) and in chemical kinetics (solid-state reactions, intercalation).

Progress in these new fields has now been brought together and critically presented by an active worker. Description of the various applications is preceded by four chapters which set out the physical

principles and operational techniques. The microscope itself is dealt with briefly, fuller accounts being readily available. Greater attention is paid to the interaction of electrons with the specimen, including radiation damage, and to the mechanism of image formation and contrast. A separate chapter is devoted to the special conditions of high resolution microscopy, where phase contrast and lattice imaging are principally involved. The design of ancillary apparatus, with particular reference to special stages, and the various methods of specimen preparation, are also surveyed.

The meat of the book, however, is comprised in the later four chapters describing the use of electron microscopy in a variety of chemical investigations. Not unnaturally, greatest space is accorded to catalysts and their reactions, to the structure of crystals (especially graphite) and to molecular crystals and radiation damage to them, all subjects in which the author has made important contributions. These chapters are very fully illustrated with micrographs, not always well reproduced in my copy of the book, and line diagrams. Polymers and proteins receive less attention here; they are adequately covered elsewhere in the literature. In spite of the blurb on the cover, there is very little on the use of the electron microscope in biochemistry and materials science, but after all Dr Fryer did not set out to write an encyclopaedia of its applications.

He has done very well within this restricted plan, and the book can be highly recommended to research chemists who wish to know what this comparatively new technique could do for them. Two reservations must be stated, however. First, the references are not so completely up-to-date as could be desired. Apart from publications from his own laboratory, there are only three or four post-1975 references. The consequent omission of a great deal of recent research, especially at high resolution, is much to be regretted and is probably ascribable to delays in printing and binding, which seem to be on the increase. My second reservation concerns the price. Little more than a decade ago we could expect a well produced scientific book to cost about £1 per 100 pages. The price of this volume works out at well over six times as much, with some sacrifice in quality. Though this may be of the same order as the increase in price of a motor-car, it outstrips the rise in chemists' salaries. Sadly, few of them will be able to afford Dr Fryer's excellent monograph, but I urge librarians in spite of equally restricted resources to get it for their shelves.

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